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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF IMMUNE RELATED DISEASES

(57) Abstract

The present invention relates to a composition containing novel proteins and methods for the diagnosis and treatment of immune related diseases.

COMPOSITIONS AND METHODS FOR THE TREATMENT OF IMMUNE RELATED DISEASES

Field of the Invention

The present invention relates to compositions and methods for the diagnosis and treatment of immune related diseases.

Background of the Invention

Immune related and inflammatory diseases are the manifestation or consequence of fairly complex, often multiple interconnected biological pathways which in normal physiology are critical to respond to insult or injury, initiate repair from insult or injury, and mount innate and acquired defense against foreign organisms. Disease or pathology occurs when these normal physiological pathways cause additional insult or injury either as directly related to the intensity of the response, as a consequence of abnormal regulation or excessive stimulation, as a reaction to self, or as a combination of these.

Though the genesis of these diseases often involves multistep pathways and often multiple different biological systems/pathways, intervention at critical points in one or more of these pathways can have an ameliorative or therapeutic effect. Therapeutic intervention can occur by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway.

Many immune related diseases are known and have been extensively studied. Such diseases include immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, *etc.*

T lymphocytes (T cells) are an important component of a mammalian immune response. T cells recognize antigens which are associated with a self-molecule encoded by genes within the major histocompatibility complex (MHC). The antigen may be displayed together with MHC molecules on the surface of antigen presenting cells, virus infected cells, cancer cells, grafts, *etc.* The T cell system eliminates these altered cells which pose a health threat to the host mammal. T cells include helper T cells and cytotoxic T cells. Helper T cells proliferate extensively following recognition of an antigen -MHC complex on an antigen presenting cell. Helper T cells also secrete a variety of cytokines, *i.e.*, lymphokines, which play a central role in the activation of B cells, cytotoxic T cells and a variety of other cells which participate in the immune response.

A central event in both humoral and cell mediated immune responses is the activation and clonal expansion of helper T cells. Helper T cell activation is initiated by the interaction of the T cell receptor (TCR) - CD3 complex with an antigen-MHC on the surface of an antigen presenting cell. This interaction mediates a cascade of biochemical events that induce the resting helper T cell to enter a cell cycle (the G0 to G1 transition) and results in the expression of a high affinity receptor for IL-2 and sometimes IL-4. The activated T cell progresses through the cycle proliferating and differentiating into memory cells or effector cells.

In addition to the signals mediated through the TCR, activation of T cells involves additional costimulation induced by cytokines released by the antigen presenting cell or through interactions with membrane bound molecules on the antigen presenting cell and the T cell. The cytokines IL-1 and IL-6 have been shown to provide a costimulatory signal. Also, the interaction between the B7 molecule expressed on the

surface of an antigen presenting cell and CD28 and CTLA-4 molecules expressed on the T cell surface effect T cell activation. Activated T cells express an increased number of cellular adhesion molecules, such as ICAM-1, integrins, VLA-4, LFA-1, CD56, *etc.*

T-cell proliferation in a mixed lymphocyte culture or mixed lymphocyte reaction (MLR) is an established indication of the ability of a compound to stimulate the immune system. In many immune responses, inflammatory cells infiltrate the site of injury or infection. The migrating cells may be neutrophilic, eosinophilic, monocytic or lymphocytic as can be determined by histologic examination of the affected tissues. Current Protocols in Immunology, ed. John E. Coligan, 1994. John Wiley & Sons, Inc.

Immune related diseases can be treated by suppressing the immune response. Using neutralizing antibodies that inhibit molecules having immune stimulatory activity would be beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

Summary of the Invention

The present invention concerns compositions and methods for the diagnosis and treatment of immune related disease in mammals, including humans. The present invention is based on the identification of proteins (including agonist and antagonist antibodies) which either stimulate or inhibit the immune response in mammals. Immune related diseases can be treated by suppressing or enhancing the immune response. Molecules that enhance the immune response stimulate or potentiate the immune response to an antigen. Molecules which stimulate the immune response can be used therapeutically where enhancement of the immune response would be beneficial. Such stimulatory molecules can also be inhibited where suppression of the immune response would be of value.

Neutralizing antibodies are examples of molecules that inhibit molecules having immune stimulatory activity and which would be beneficial in the treatment of immune related and inflammatory diseases. Molecules which inhibit the immune response can also be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

Accordingly, the PRO polypeptides and anti-PRO antibodies and fragments thereof are useful for the diagnosis and/or treatment (including prevention) of immune related diseases. Antibodies which bind to stimulatory proteins are useful to suppress the immune system and the immune response. Antibodies which bind to inhibitory proteins are useful to stimulate the immune system and the immune response. The PRO polypeptides and anti-PRO antibodies also useful to prepare medicines and medicaments for the treatment of immune related and inflammatory diseases.

In one embodiment, the invention provides for isolated nucleic acid molecules comprising nucleotide sequences that encodes a PRO polypeptide.

In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about

87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity, alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule comprising the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity,

alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity, alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein, or (b) the complement of the DNA molecule of (a).

In another aspect, the invention provides for isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide with is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptides are disclosed herein. Therefore, soluble extracellular domains of the herein described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO polypeptide antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160 nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length, alternatively at least about 1000 nucleotides in length, alternatively at least about 1500 nucleotide in length, alternatively at least about 2000 nucleotides in length, alternatively at least about 2500 nucleotide in length, alternatively at least about 3000 nucleotide in length, alternatively at least about 4000 nucleotide in length, alternatively at least about 5000 nucleotides in length, or more, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length. It is noted that novel fragments of a nucleotide sequence encoding the respective PRO polypeptide may be determined in a routine manner by aligning the respective nucleotide encoding a PRO polypeptide with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which nucleotide sequence fragment(s) are novel. All such nucleotide sequences encoding the respective PRO polypeptides are contemplated herein. Also contemplated are the nucleotide molecules which encode fragments of the PRO polypeptides, preferably those polypeptide fragments that comprise a binding site for an anti-PRO polypeptide antibody.

In another embodiment, the invention provides isolated PRO polypeptides encoded by any of the isolated nucleic acid sequences hereinabove identified.

In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity, alternatively at least about 99% amino acid sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity, alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence scoring at least about 80% positives, alternatively at least about 81% positives, alternatively at least about 82% positives, alternatively at least about 83% positives, alternatively at least about 84% positives, alternatively at least about 85% positives, alternatively at least about 86% positives, alternatively at least about 87% positives, alternatively at least about 88% positives, alternatively at least about 89% positives, alternatively at least about 90% positives, alternatively at least about 91% positives, alternatively at least about 92% positives, alternatively at least about 93% positives, alternatively at least about 94% positives, alternatively at least about 95% positives, alternatively at least about 96% positives, alternatively at least about

97% positives, alternatively at least about 98% positives, alternatively at least about 99% positives when compared with the amino acid sequence of a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as hereinbefore described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the same from the cell culture.

In another aspect, the invention provides an isolated PRO polypeptide which is either transmembrane-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

In another embodiment, the invention provides vectors comprising DNA encoding any of the PRO polypeptides. Host cells comprising any such vector are also provided. By way of example, the host cells may be CHO cells, *E. coli* or yeast. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptides and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Examples of such chimeric molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

In yet another embodiment, the invention concerns agonists and antagonists of the PRO polypeptides that mimic or inhibit one or more functions or activities of the PRO polypeptides. In a particular embodiment, the agonist or antagonist is an antibody that binds to the PRO polypeptides or a small molecule.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody. In one aspect, the present invention concerns an isolated antibody which binds a PRO polypeptide. In another aspect, the antibody mimics the activity of a PRO polypeptide (an agonist antibody) or conversely the antibody inhibits or neutralizes the activity of a PRO polypeptide (an antagonist antibody). In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a monoclonal antibody, a single-chain antibody, or an anti-idiotypic

antibody.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprises contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native sequence PRO polypeptide.

In another embodiment, the invention concerns a composition of matter containing PRO polypeptide or an agonist or antagonist antibody which binds the polypeptide in admixture with a carrier or excipient. In one aspect, the composition contains a therapeutically effective amount of the peptide or antibody. In another aspect, when the composition contains an immune stimulating molecule, the composition is useful for: (a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) stimulating or enhancing an immune response in a mammal in need thereof, or (c) increasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen. In a further aspect, when the composition contains an immune inhibiting molecule, the composition is useful for: (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) inhibiting or reducing an immune response in a mammal in need thereof, or (c) decreasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen. In another aspect, the composition contains a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

In another embodiment, the invention concerns the use of the polypeptides and antibodies of the invention to prepare a composition or medicament which has the uses described above.

In a further embodiment, the invention concerns nucleic acid encoding an anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody, and vectors and recombinant host cells comprising such nucleic acid. In a still further embodiment, the invention concerns a method for producing such an antibody by culturing a host cell transformed with nucleic acid encoding the antibody under conditions such that the antibody is expressed, and recovering the antibody from the cell culture.

In a further embodiment, the invention concerns an isolated nucleic acid molecule that hybridizes to the a nucleic acid molecule encoding a PRO polypeptide, or the complement thereof. The nucleic acid preferably is DNA, and hybridization preferably occurs under stringent conditions. Such nucleic acid molecules can act as antisense molecules of the amplified genes identified herein, which, in turn, can find use in the modulation of the respective amplified genes, or as antisense primers in amplification reactions. Furthermore, such sequences can be used as part of ribozyme and/or triple helix sequence which, in turn, may be used in regulation of the amplified genes.

In another embodiment, the invention concerns a method for determining the presence of a PRO polypeptide comprising exposing a cell suspected of containing and/or expressing the polypeptide to an anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody and determining binding of the antibody to the cell.

In yet another embodiment, the present invention concerns a method of diagnosing an immune related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

In another embodiment, the present invention concerns a method of diagnosing an immune disease in a mammal, comprising (a) contacting an anti-PRO polypeptide antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and the respective PRO polypeptide, respectively, in the test sample; wherein the formation of said complex is indicative of the presence or absence of said disease. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence or absence of an immune disease in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually obtained from an individual suspected of having a deficiency or abnormality of the immune system.

In another embodiment, the present invention concerns a diagnostic kit, containing an anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody and a carrier (*e.g.*, a buffer) in suitable packaging. The kit preferably contains instructions for using the antibody to detect the PRO

polypeptide.

In a further embodiment, the invention concerns an article of manufacture, comprising:

a container;

an instruction on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for stimulating or inhibiting an immune response in a mammal, the instruction on the container indicates that the composition can be used to treat an immune related disease, and the active agent in the composition is an agent stimulating or inhibiting the expression and/or activity of the PRO polypeptide. In a preferred aspect, the active agent is a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide or an anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody.

A further embodiment is a method for identifying a compound capable of inhibiting the expression and/or activity of a PRO polypeptide by contacting a candidate compound with a PRO polypeptide under conditions and for a time sufficient to allow these two components to interact. In a specific aspect, either the candidate compound or the PRO polypeptide is immobilized on a solid support. In another aspect, the non-immobilized component carries a detectable label.

Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as hereinbefore described, or an anti-PRO antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

Brief Description of the Drawings

Figure 1 shows DNA29101-1276 (SEQ ID NO:1).

Figure 2 shows the native sequence PRO200 polypeptide UNQ174 (SEQ ID NO:2).

Figure 3 shows DNA30871-1157 (SEQ ID NO:11).

Figure 4 shows the native sequence partial length PRO204 polypeptide UNQ178 (SEQ ID NO:12).

Figure 5 shows DNA30942-1134 (SEQ ID NO:13).

Figure 6 shows the native sequence PRO212 polypeptide UNQ186 (SEQ ID NO:14).
Figure 7 shows DNA33087-1158 (SEQ ID NO:18).
Figure 8 shows the native sequence PRO216 polypeptide UNQ190 (SEQ ID NO:19).
Figure 9 shows DNA33460-1166 (SEQ ID NO:20).
Figure 10 shows the native sequence PRO226 polypeptide UNQ200 (SEQ ID NO:21).
Figure 11 shows DNA34387-1138 (SEQ ID NO:25).
Figure 12 shows the native sequence PRO240 polypeptide UNQ214 (SEQ ID NO:26).
Figure 13 shows DNA35558-1167 (SEQ ID NO:30).
Figure 14 shows the native sequence PRO235 polypeptide UNQ209 (SEQ ID NO:31).
Figure 15 shows DNA35638-1141 (SEQ ID NO:35).
Figure 16 shows the native sequence PRO245 polypeptide UNQ219 (SEQ ID NO:36).
Figure 17 shows DNA35916-1161 (SEQ ID NO:40).
Figure 18 shows the native sequence PRO172 polypeptide UNQ146 (SEQ ID NO:41).
Figure 19 shows DNA39523-1192 (SEQ ID NO:45).
Figure 20 shows the native sequence PRO273 polypeptide UNQ240 (SEQ ID NO:46).
Figure 21 shows DNA40620-1183 (SEQ ID NO:50).
Figure 22 shows the native sequence PRO272 polypeptide UNQ239 (SEQ ID NO:51).
Figure 23 shows DNA40982-1235 (SEQ ID NO:56).
Figure 24 shows the native sequence PRO332 polypeptide UNQ293 (SEQ ID NO:57).
Figure 25 shows DNA44184-1319 (SEQ ID NO:61).
Figure 26 shows the native sequence PRO526 polypeptide UNQ330 (SEQ ID NO:62).
Figure 27 shows DNA44205-1285 (SEQ ID NO:66).
Figure 28 shows the native sequence PRO701 polypeptide UNQ365 (SEQ ID NO:67).
Figure 29 shows DNA45410-1250 (SEQ ID NO:71).
Figure 30 shows the native sequence PRO361 polypeptide UNQ316 (SEQ ID NO:72).
Figure 31 shows DNA45416-1251 (SEQ ID NO:79).
Figure 32 shows the native sequence PRO362 polypeptide UNQ317 (SEQ ID NO:80).
Figure 33 shows DNA45419-1252 (SEQ ID NO:86).
Figure 34 shows the native sequence PRO363 polypeptide UNQ318 (SEQ ID NO:87).
Figure 35 shows DNA47365-1206 (SEQ ID NO:91).
Figure 36 shows the native sequence PRO364 polypeptide UNQ319 (SEQ ID NO:92).
Figure 37 shows DNA47470-1130 (SEQ ID NO:101).
Figure 38 shows the native sequence PRO356 polypeptide UNQ313 (SEQ ID NO:102).
Figure 39 shows DNA48314-1320 (SEQ ID NO:106).
Figure 40 shows the native sequence PRO531 polypeptide UNQ332 (SEQ ID NO:107).
Figure 41 shows DNA49435-1219 (SEQ ID NO:111).
Figure 42 shows the native sequence PRO533 polypeptide UNQ334 (SEQ ID NO:112).
Figure 43 shows DNA50921-1458 (SEQ ID NO:116).
Figure 44 shows the native sequence PRO1083 polypeptide UNQ540 (SEQ ID NO:117).
Figure 45 shows DNA53974-1401 (SEQ ID NO:123).

Figure 46 shows the native sequence PRO865 polypeptide UNQ434 (SEQ ID NO:124).
Figure 47 shows DNA54228-1366 (SEQ ID NO:133).
Figure 48 shows the native sequence PRO770 polypeptide UNQ408 (SEQ ID NO:134).
Figure 49 shows DNA54231-1366 (SEQ ID NO:139).
Figure 50 shows the native sequence PRO769 polypeptide UNQ407 (SEQ ID NO:140).
Figure 51 shows DNA56405-1357 (SEQ ID NO:141).
Figure 52 shows the native sequence PRO788 polypeptide UNQ430 (SEQ ID NO:142).
Figure 53 shows DNA57033-1403 (SEQ ID NO:143).
Figure 54 shows the native sequence PRO1114 polypeptide UNQ557 (SEQ ID NO:144).
Figure 55 shows DNA57690-1374 (SEQ ID NO:145).
Figure 56 shows the native sequence PRO1007 polypeptide UNQ491 (SEQ ID NO:146).
Figure 57 shows DNA59220-1514 (SEQ ID NO:147).
Figure 58 shows the native sequence PRO1184 polypeptide UNQ598 (SEQ ID NO:148).
Figure 59 shows DNA59294-1381 (SEQ ID NO:149).
Figure 60 shows the native sequence PRO1031 polypeptide UNQ516 (SEQ ID NO:150).
Figure 61 shows DNA59776-1600 (SEQ ID NO:151).
Figure 62 shows the native sequence PRO1346 polypeptide UNQ701 (SEQ ID NO:152).
Figure 63 shows DNA59849-1504 (SEQ ID NO:156).
Figure 64 shows the native sequence PRO1155 polypeptide UNQ585 (SEQ ID NO:157).
Figure 65 shows DNA60775-1532 (SEQ ID NO:158).
Figure 66 shows the native sequence PRO1250 polypeptide UNQ633 (SEQ ID NO:159).
Figure 67 shows DNA61873-1574 (SEQ ID NO:160).
Figure 68 shows the native sequence PRO1312 polypeptide UNQ678 (SEQ ID NO:161).
Figure 69 shows DNA62814-1521 (SEQ ID NO:162).
Figure 70 shows the native sequence PRO1192 polypeptide UNQ606 (SEQ ID NO:163).
Figure 71 shows DNA64885-1529 (SEQ ID NO:167).
Figure 72 shows the native sequence PRO1246 polypeptide UNQ630 (SEQ ID NO:168).
Figure 73 shows DNA65404-1551 (SEQ ID NO:169).
Figure 74 shows the native sequence PRO1283 polypeptide UNQ653 (SEQ ID NO:170).
Figure 75 shows DNA65412-1523 (SEQ ID NO:177).
Figure 76 shows the native sequence PRO1195 polypeptide UNQ608 (SEQ ID NO:178).
Figure 77 shows DNA66675-1587 (SEQ ID NO:179).
Figure 78 shows the native sequence PRO1343 polypeptide UNQ698 (SEQ ID NO:180).
Figure 79 shows DNA68864-1629 (SEQ ID NO:184).
Figure 80 shows the native sequence PRO1418 polypeptide UNQ732 (SEQ ID NO:185).
Figure 81 shows DNA68872-1620 (SEQ ID NO:186).
Figure 82 shows the native sequence PRO1387 polypeptide UNQ722 (SEQ ID NO:187).
Figure 83 shows DNA68874-1622 (SEQ ID NO:188).
Figure 84 shows the native sequence PRO1410 polypeptide UNQ728 (SEQ ID NO:189).
Figure 85 shows DNA76400-2528 (SEQ ID NO:190).

Figure 86 shows the native sequence PRO1917 polypeptide UNQ900 (SEQ ID NO:191).
Figure 87 shows DNA77624-2515 (SEQ ID NO:192).
Figure 88 shows the native sequence PRO1868 polypeptide UNQ859 (SEQ ID NO:193).
Figure 89 shows DNA30868-1156 (SEQ ID NO:228).
Figure 90 shows the partial native sequence PRO205 polypeptide UNQ179 (SEQ ID NO:229).
Figure 91 shows DNA36638-1056 (SEQ ID NO:230).
Figure 92 shows the native sequence PRO21 polypeptide UNQ21 (SEQ ID NO:231).
Figure 93 shows DNA38260-1180 (SEQ ID NO:232).
Figure 94 shows the native sequence PRO269 polypeptide UNQ236 (SEQ ID NO:233).
Figure 95 shows DNA40592-1242 (SEQ ID NO:240).
Figure 96 shows the native sequence PRO344 polypeptide UNQ303 (SEQ ID NO:241).
Figure 97 shows DNA41374-1312 (SEQ ID NO:248).
Figure 98 shows the partial length native sequence PRO333 polypeptide UNQ294 (SEQ ID NO:249).
Figure 99 shows DNA44194-1317 (SEQ ID NO:250).
Figure 100 shows the native sequence PRO381 polypeptide UNQ322 (SEQ ID NO:251).
Figure 101 shows DNA53517-1366 (SEQ ID NO:255).
Figure 102 shows the native sequence PRO720 polypeptide UNQ388 (SEQ ID NO:256).
Figure 103 shows DNA53971-1359 (SEQ ID NO:257).
Figure 104 shows the native sequence PRO866 polypeptide UNQ435 (SEQ ID NO:258).
Figure 105 shows DNA53987-1438 (SEQ ID NO:266).
Figure 106 shows the native sequence PRO840 polypeptide UNQ433 (SEQ ID NO:267).
Figure 107 shows DNA57700-1408 (SEQ ID NO:268).
Figure 108 shows the native sequence PRO982 polypeptide UNQ483 (SEQ ID NO:269).
Figure 109 shows DNA59620-1463 (SEQ ID NO:270).
Figure 110 shows the native sequence PRO836 polypeptide UNQ545 (SEQ IDNO:271).
Figure 111 shows DNA60627-1508 (SEQ ID NO:272).
Figure 112 shows the native sequence PRO1159 polypeptide UNQ589 (SEQ ID NO:273).
Figure 113 shows DNA64890-1612 (SEQ ID NO:274).
Figure 114 shows the native sequence PRO1358 polypeptide UNQ707 (SEQ ID NO:275).
Figure 115 shows DNA66659-1593 (SEQ ID NO:276).
Figure 116 shows the native sequence PRO1325 polypeptide UNQ685 (SEQ ID NO:277).
Figure 117 shows DNA66667-1596 (SEQ ID NO:278).
Figure 118 shows the native sequence PRO1338 polypeptide UNQ693 (SEQ ID NO:279).
Figure 119 shows DNA68818-2536 (SEQ ID NO:280).
Figure 120 shows the native sequence PRO1434 polypeptide UNQ739 (SEQ ID NO:281).
Figure 121 shows DNA84210-2576 (SEQ ID NO:285).
Figure 122 shows the native sequence PRO4333 polypeptide UNQ1888 (SEQ ID NO:286).
Figure 123 shows DNA92218-2554 (SEQ ID NO:292).
Figure 124 shows the native sequence PRO4302 polypeptide UNQ1866 (SEQ ID NO:293).
Figure 125 shows DNA96878-2626 (SEQ ID NO:294).

Figure 126 shows the native sequence PRO4430 polypeptide UNQ1947 (SEQ ID NO:295).

Figure 127 shows DNA98853-1739 (SEQ ID NO:296).

Figure 128 shows the native sequence PRO5727 polypeptide UNQ2448 (SEQ ID NO:297).

Detailed Description of the Preferred Embodiments

I. Definitions

The terms "PRO polypeptide(s)" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (*i.e.*, "PRO/number" or more particularly, PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727) refers to particular polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided as an actual numerical designation (*e.g.*, as described above) as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence PRO polypeptide(s)" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO/number polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide(s)" specifically encompasses naturally-occurring truncated or secreted forms of the specific PRO/number polypeptide (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO polypeptides disclosed herein are mature or full-length native sequence polypeptides comprising the full-length amino acids sequences shown in the accompanying figures. Start and stop codons are shown in bold font and underlined in the figures. However, while the PRO/number polypeptides disclosed in the accompanying figures are shown to begin with methionine residues designated herein as amino acid position 1 in the figures, it is conceivable and possible that other methionine residues located either upstream or downstream from the amino acid position 1 in the figures may be employed as the starting amino acid residue for the PRO polypeptides.

The "PRO polypeptide(s) extracellular domain" or "ECD" refers to a form of the said polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domains identified for the PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified herein.

Optionally, therefore, an extracellular domain of a PRO polypeptide may contain from about 5 or fewer amino acids on either side of the transmembrane domain/extracellular domain boundary as identified in the Examples or specification and such polypeptides, with or without the associated signal peptide, and nucleic acid encoding them, are contemplated by the present invention.

The approximate location of the "signal peptides" of the various PRO/number PRO polypeptides disclosed herein are shown in the present specification and/or the accompanying figures. It is noted, however, that the C-terminal boundary of a signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art for identifying that type of amino acid sequence element (*e.g.*, Nielsen *et al.*, *Prot. Eng.* 10:1-6 (1997) and von Heinje *et al.*, *Nucl. Acids. Res.* 14:4683-4690 (1986)). Moreover, it is also recognized that, in some cases, cleavage of a signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These mature polypeptides, where the signal peptide is cleaved within no more than about 5 amino acids on either side of the C-terminal boundary of the signal peptide as identified herein, and the polynucleotides encoding them, are contemplated by the present invention.

A "PRO polypeptide variant", "PRO/number variant" or "PRO variant" means an active PRO polypeptide as defined herein (*e.g.*, below) having at least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity, alternatively at least about 99% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, PRO polypeptide variants are at least about 10 amino acids in length, alternatively at least about 20 amino acids in length, alternatively at least about 30 amino acids in length, alternatively at least about 40 amino acids in length, alternatively at least about 50 amino acids in length,

alternatively at least about 60 amino acids in length, alternatively at least about 70 amino acids in length, alternatively at least about 80 amino acids in length, alternatively at least about 90 amino acids in length, alternatively at least about 100 amino acids in length, alternatively at least about 150 amino acids in length, alternatively at least about 200 amino acids in length, alternatively at least about 300 amino acids in length, alternatively at least about 400 amino acids on length, alternatively at least about 500 amino acids in length, alternatively at least about 600 amino acids in length, alternatively at least about 700 amino acids in length, alternatively at least about 800 amino acids in length, alternatively at least about 900 amino acids in length, alternatively at least about 1000 amino acids in length, alternatively at least bout 1200 amino acids in length, alternatively at least about 1400 amino acids in length, alternatively at least about 1500 amino acids in length or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific PRO/number polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Tables 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. As examples of % amino acid sequence identity calculations using this method, Tables 2

and 3 demonstrate how to calculate the % amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO", wherein "PRO" represents the amino acid sequence of a hypothetical PRO/number polypeptide of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, and "X, "Y" and "Z" each represent different hypothetical amino acid residues.

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % amino acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul *et al., Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, *i.e.*, the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native sequence PRO polypeptide and the comparison amino acid sequence of interest (*i.e.*, the sequence against which the PRO polypeptide is being compared - which may be a PRO polypeptide variant) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest. For example, in the statement "a polypeptide comprising an amino acid sequence A which has or having at least 80% amino acid sequence identity to the amino acid sequence B", the amino acid sequence A is the comparison amino acid sequence of interest and the amino acid sequence B is the amino acid sequence of the PRO polypeptide of interest.

Percent amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al., Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from "http://www.ncbi.nlm.gov" or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide sequence encoding: (1) a full-length native sequence PRO polypeptide as disclosed herein; (2) a full-length native sequence PRO polypeptide lacking the signal peptide as disclosed herein; (3) an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or (4) any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO polypeptide variant polynucleotide will have at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity, alternatively at least about 99% nucleic acid sequence identity with (1) a nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, (2) a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, (3) an extracellular domain of a PRO polypeptide sequence, with or without the signal sequence, as disclosed herein or (4) any other fragment of a full-length PRO polypeptide on sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO polypeptide variant polynucleotides are at least about 30 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 210 nucleotides in length, alternatively at least about 240 nucleotides in length, alternatively at least about 270 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length, alternatively at least about 1000 nucleotides in length, alternatively at least about 1200 nucleotides in length, alternatively at least about 1400 nucleotides in length, alternatively at least about 1600 nucleotides in length, alternatively at least about 1800 nucleotides in length, alternatively at least about 2000 nucleotides in length, alternatively at least about 2500 nucleotides in length, alternatively at least about 3000 nucleotides in length, alternatively at least about 3500 nucleotides in length, alternatively at least about 4000 nucleotides, alternatively at least about 5000 nucleotides or more.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if

necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For purposes herein, however, % nucleic acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for nucleic acid sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction W/Z

where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 4 and 5, demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA", wherein "PRO-DNA" represents a hypothetical PRO polypeptide - encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, and "N", "L" and "V" each represent different hypothetical nucleotides.

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % nucleic acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul *et al., Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, *i.e.*, the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide - encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide - encoding nucleic acid (*i.e.*, the reference sequence) and the comparison nucleic acid molecule of interest (*i.e.*, the sequence against which the PRO polypeptide - encoding nucleic acid

molecule of interest is being compared - which may be a PRO variant polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO reference sequence. For example, in the statement "an isolated nucleic acid molecule comprising a nucleic acid sequence A which has or having at least 80% nucleic acid sequence identity to the nucleic acid sequence B", the nucleic acid sequence A is the comparison nucleic acid molecule of interest and the nucleic acid sequence B is the nucleic acid sequence of the PRO polypeptide - encoding nucleic acid molecule of interest.

Percent nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al., Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from "http://www.ncbi.nlm.nih.gov" or otherwise obtained from the National Institute of Heath, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction W/Z

where W is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding a full-length PRO polypeptides as disclosed herein. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

The term "positives", in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (*e.g.*, as a result of conservative substitutions, see Table 6 below). For purposes herein, the % value of positives is determined by dividing (a) the number of amino acid residues scoring a positive value between the PRO polypeptide sequence of interest having a sequence derived from a native sequence PRO polypeptide and the comparison amino acid sequence of interest (*i.e.*, the amino acid sequence against which the PRO polypeptide sequence is being compared) as determined in the BLOSUM62 matrix of WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest.

Unless specifically stated otherwise, the % value of positives is calculated as described in the immediately preceding paragraph. However, in the context of the amino acid sequence identity comparisons performed as described for ALIGN-2 and NCBI-BLAST-2 above, includes amino acid residues in the

sequences compared that are not only identical, but also those that have similar properties. Amino acid residues that score a positive value to an amino acid residue of interest are those that are either identical to the amino acid residue of interest or are a preferred substitution (as defined in Table I below) of the amino acid residue of interest.

For amino acid sequence comparisons using ALIGN-2 or NCBI-BLAST2, the % value of positives of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % positives to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scoring a positive value as defined above by the sequence alignment program ALIGN-2 or NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % positives of A to B will not equal the % positives of B to A.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO polypeptide in its natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide - encoding nucleic acid or other polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. An isolated polypeptide-encoding nucleic acid molecule is other than in the context or setting in which it is found in nature. Isolated polypeptide - encoding nucleic acids therefore are distinguished from the polypeptide - encoding nucleic acid molecule existing in natural cells. However, an isolated PRO polypeptide - encoding nucleic acid molecule includes the same contained in cells that ordinarily express the specific polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a

polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide: a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence: or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous. and. in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist. the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length. washing temperature. and salt concentration. In general. longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence. the higher the relative temperature which can be used. As a result. it follows that higher relative temperatures would tend to make the reaction conditions more stringent. while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions. see Ausubel *et al., Current Protocols in Molecular Biology*. Wiley Interscience Publishers. (1995).

"Stringent conditions" or "high stringency conditions." as defined herein. may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride. 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl. 0.075 M sodium citrate). 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate. 5 x Denhardt's solution. sonicated salmon sperm DNA (50 µg/ml). 0.1% SDS. and 10% dextran sulfate at 42°C. with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C. followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook *et al., Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Press. 1989. and include the use of washing solution and hybridization conditions (*e.g.*. temperature. ionic strength and %SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide. 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6). 5 x Denhardt's solution. 10% dextran sulfate. and 20 µg/mL denatured sheared salmon sperm DNA. followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature. ionic strength, *etc.*. as necessary to accommodate factors such as probe length and the like.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same general structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense and specifically covers. without limitation, intact monoclonal

antibodies (including agonist, antagonist and neutralizing antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies) formed from at least two intact antibodies, single chain antibodies binding the epitopes specific to the PRO polypeptide and antibody fragments so long as they exhibit the desired biological activity. An anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody is an antibody which immunologically binds to a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727, respectively, polypeptide. The antibody may bind to any domain of the PRO polypeptide which may be contacted by the antibody. For example, the antibody may bind to any extracellular domain of the polypeptide and when the entire polypeptide is secreted, to any domain on the polypeptide which is available to the antibody for binding.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three or four segments called "complementarity-determining regions" (CDRs) or "hypervariable regions" in both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four or five FR regions, largely adopting a β–sheet configuration, connected by the CDRs, which form loops connecting, and in some cases forming part of, the β–sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain,

contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, NIH Publ. No.91-3242, Vol. I, pages 647-669 (1991)). There are at least two techniques for determining the extent of the CDRs: (1) An approach based on the extent of cross-species sequence variability (*i.e.*, Kabat *et al.*, *Sequences of Proteins of Immunological Interest* (National Institute of Health, Bethesda, MD); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Chothia, C. *et al.*, (1989), *Nature* 342: 877). Moreover, CDR's can also be defined using a hybrid approach incorporating the residues identified by both of the previous techniques. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata *et al.*, *Protein Eng.* 8 (10):1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen- binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256: 495 [1975], or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 [1991] and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example. See also U.S Patent Nos. 5,750,373, 5,571,698, 5,403,484 and 5,223,409 which describe the preparation of antibodies using phagemid and phage vectors.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 [1984]).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332:323-329 [1988]; and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992). The humanized antibody includes a "primatized"antibody where the antigen-binding region of the antibody is derived from an antibody produced

by immunizing macaque monkeys with the antigen of interest. Antibodies containing residues from Old World monkeys are also possible within the invention. See, for example, U.S. Patent Nos. 5,658,570; 5,693,780; 5,681,722; 5,750,105; and 5,756,096.

Antibodies and fragments thereof in this invention also include "affinity matured" antibodies in which an antibody is altered to change the amino acid sequence of one or more of the CDR regions and/or the framework regions to alter the affinity of the antibody or fragment thereof for the antigen to which it binds. Affinity maturation may result in an increase or in a decrease in the affinity of the matured antibody for the antigen relative to the starting antibody. Typically, the starting antibody will be a humanized, human, chimeric or murine antibody and the affinity matured antibody will have a higher affinity than the starting antibody. During the maturation process, one or more of the amino acid residues in the CDRs or in the framework regions are changed to a different residue using any standard method. Suitable methods include point mutations using well known cassette mutagenesis methods (Wells *et al.*, 1985. *Gene* 34:315) or oligonucleotide mediated mutagenesis methods (Zoller *et al.*, 1987, *Nucleic Acids Res.* 10:6487-6504). Affinity maturation may also be performed using known selection methods in which many mutations are produced and mutants having the desired affinity are selected from a pool or library of mutants based on improved affinity for the antigen or ligand. Known phage display techniques can be conveniently used in this approach. See, for example, U.S. 5,750,373; U.S. 5,223,409, *etc.*

Human antibodies are also with in the scope of the antibodies of the invention. Human antibodies can be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147 (1):86-95 (1991); U. S. 5,750, 373]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *Bio/Technology* 10, 779-783 (1992); Lonberg *et al.*, *Nature* 368 856-859 (1994); Morrison, *Nature* 368, 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995).

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain

and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the compound, *e.g.*, antibody or polypeptide, so as to generate a "labelled" compound. The label may be detectable by itself (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the compound of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*, controlled pore glass), polysaccharides (*e.g.*, agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.*, an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or otherwise contributes to a morbidity in the mammal. Also included are diseases in which stimulation or intervention of the immune response has an ameliorative effect on progression of the disease. Included within this term are immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, *etc.*

The term "T cell mediated" disease means a disease in which T cells directly or indirectly mediate or otherwise contribute to a morbidity in a mammal. The T cell mediated disease may be associated with cell mediated effects, lymphokine mediated effects, *etc.*, and even effects associated with B cells if the B cells are stimulated, for example, by the lymphokines secreted by T cells.

Examples of immune-related and inflammatory diseases, some of which are immune or T cell mediated, which can be treated according to the invention include systemic lupus erythematosis, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease. Infectious diseases including viral

diseases such as AIDS (HIV infection), hepatitis A, B, C, D, and E, herpes, *etc.*, bacterial infections, fungal infections, protozoal infections and parasitic infections.

"Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. In treatment of an immune related disease, a therapeutic agent may directly decrease or increase the magnitude of response of a component of the immune response, or render the disease more susceptible to treatment by other therapeutic agents, *e.g.*, antibiotics, antifungals, anti-inflammatory agents, chemotherapeutics, *etc.*

The term "effective amount" is at least the minimum concentration or amount of a PRO polypeptide and/or agonist/antagonist which causes, induces or results in either a detectable improvement in a component of the immune response in mammals as measured in an *in vitro* assay. For example, an increase or decrease in the proliferation of T-cells and/or vascular permeability as measured in Examples provided herein. Furthermore, a "therapeutically effective amount" is the minimum concentration or amount of a PRO polypeptide and/or agonist/antagonist which would be effective in at least attenuating a pathology (increasing or decreasing as the case may be) a component of the immune response in mammals, the results of which effects a treatment as defined in the previous paragraph.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

The "pathology" of an immune related disease includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, antibody production, auto-antibody production, complement production and activation, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of any inflammatory or immunological response, infiltration of inflammatory cells (neutrophilic, eosinophilic, monocytic, lymphocytic) into tissue spaces, *etc.*

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cattle, pigs, apes, hamsters, ferrets, cats, *etc.* Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or

sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.*, I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, *e.g.*, paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, France), toxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), melphalan and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine

includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

"Active" or "activity" in the context of variants of the PRO polypeptide refers to form(s) of proteins of the invention which retain the biologic and/or the ability to induce the production of an antibody against an antigenic epitope possessed by the PRO polypeptide. More specifically, "biological activity" refers to a biological function (either inhibitory or stimulatory) caused by a native sequence or naturally-occurring PRO polypeptide. Even more specifically, "biological activity" in the context of an antibody or another molecule that can be identified by the screening assays disclosed herein (*e.g.*, an organic or inorganic small molecule, peptide, *etc.*) can be the ability of such molecules to induce or inhibit infiltration of inflammatory cells into a tissue, to stimulate or inhibit T-cell proliferation or activation, to stimulate or inhibit cytokine release by cells or to increase or decrease vascular permeability. Another specific biological activity is the increased vascular permeability or the inhibition thereof.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native sequence PRO polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics or amplifies a biological activity of a native sequence PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, small organic molecules, *etc.* Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the same.

A "small molecule" is defined herein to have a molecular weight below about 600 daltons, and is generally an organic compound.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (optionally including a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a

ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

Table 1

```
/*
 *
 * C-C increased from 12 to 15
 * Z is average of EQ
 * B is average of ND
 * match with stop is _M: stop-stop = 0; J (joker) match = 0
 */
#define  _M      -8      /* value of a match with a stop */

int      _day[26][26] = {
/*     A  B  C  D  E  F  G  H  I  J  K  L  M  N  O  P  Q  R  S  T  U  V  W  X  Y  Z */
/* A */  { 2, 0,-2, 0, 0,-4, 1,-1,-1, 0,-1,-2,-1, 0, _M, 1, 0,-2, 1, 1, 0, 0,-6, 0,-3, 0},
/* B */  { 0, 3,-4, 3, 2,-5, 0, 1,-2, 0, 0,-3,-2, 2, _M,-1, 1, 0, 0, 0, 0,-2,-5, 0,-3, 1},
/* C */  {-2,-4,15,-5,-5,-4,-3,-3,-2, 0,-5,-6,-5,-4, _M,-3,-5,-4, 0,-2, 0,-2,-8, 0, 0,-5},
/* D */  { 0, 3,-5, 4, 3,-6, 1, 1,-2, 0, 0,-4,-3, 2, _M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 2},
/* E */  { 0, 2,-5, 3, 4,-5, 0, 1,-2, 0, 0,-3,-2, 1, _M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 3},
/* F */  {-4,-5,-4,-6,-5, 9,-5,-2, 1, 0,-5, 2, 0,-4, _M,-5,-5,-4,-3,-3, 0,-1, 0, 0, 7,-5},
/* G */  { 1, 0,-3, 1, 0,-5, 5,-2,-3, 0,-2,-4,-3, 0, _M,-1,-1,-3, 1, 0, 0,-1,-7, 0,-5, 0},
/* H */  {-1, 1,-3, 1, 1,-2,-2, 6,-2, 0, 0,-2,-2, 2, _M, 0, 3, 2,-1,-1, 0,-2,-3, 0, 0, 2},
/* I */  {-1,-2,-2,-2,-2, 1,-3,-2, 5, 0,-2, 2, 2,-2, _M,-2,-2,-2,-1, 0, 0, 4,-5, 0,-1,-2},
/* J */  { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */  {-1, 0,-5, 0, 0,-5,-2, 0,-2, 0, 5,-3, 0, 1, _M,-1, 1, 3, 0, 0, 0,-2,-3, 0,-4, 0},
/* L */  {-2,-3,-6,-4,-3, 2,-4,-2, 2, 0,-3, 6, 4,-3, _M,-3,-2,-3,-3,-1, 0, 2,-2, 0,-1,-2},
/* M */  {-1,-2,-5,-3,-2, 0,-3,-2, 2, 0, 0, 4, 6,-2, _M,-2,-1, 0,-2,-1, 0, 2,-4, 0,-2,-1},
/* N */  { 0, 2,-4, 2, 1,-4, 0, 2,-2, 0, 1,-3,-2, 2, _M,-1, 1, 0, 1, 0, 0,-2,-4, 0,-2, 1},
/* O */  {_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M, 0,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M},
/* P */  { 1,-1,-3,-1,-1,-5,-1, 0,-2, 0,-1,-3,-2,-1, _M, 6, 0, 0, 1, 0, 0,-1,-6, 0,-5, 0},
/* Q */  { 0, 1,-5, 2, 2,-5,-1, 3,-2, 0, 1,-2,-1, 1, _M, 0, 4, 1,-1,-1, 0,-2,-5, 0,-4, 3},
/* R */  {-2, 0,-4,-1,-1,-4,-3, 2,-2, 0, 3,-3, 0, 0, _M, 0, 1, 6, 0,-1, 0,-2, 2, 0,-4, 0},
/* S */  { 1, 0, 0, 0, 0,-3, 1,-1,-1, 0, 0,-3,-2, 1, _M, 1,-1, 0, 2, 1, 0,-1,-2, 0,-3, 0},
/* T */  { 1, 0,-2, 0, 0,-3, 0,-1, 0, 0, 0,-1,-1, 0, _M, 0,-1,-1, 1, 3, 0, 0,-5, 0,-3, 0},
/* U */  { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */  { 0,-2,-2,-2,-2,-1,-1,-2, 4, 0,-2, 2, 2,-2, _M,-1,-2,-2,-1, 0, 0, 4,-6, 0,-2,-2},
/* W */  {-6,-5,-8,-7,-7, 0,-7,-3,-5, 0,-3,-2,-4,-4, _M,-6,-5, 2,-2,-5, 0,-6,17, 0, 0,-6},
/* X */  { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */  {-3,-3, 0,-4,-4, 7,-5, 0,-1, 0,-4,-1,-2,-2, _M,-5,-4,-4,-3,-3, 0,-2, 0, 0,10,-4},
/* Z */  { 0, 1,-5, 2, 3,-5, 0, 2,-2, 0, 0,-2,-1, 1, _M, 0, 3, 0, 0, 0, 0,-2,-6, 0,-4, 4}
};
```

Table 1 (cont')

```
/*
 */
#include <stdio.h>
#include <ctype.h>

#define  MAXJMP     16      /* max jumps in a diag */
#define  MAXGAP     24      /* don't continue to penalize gaps larger than this */
#define  JMPS       1024    /* max jmps in an path */
#define  MX         4       /* save if there's at least MX-1 bases since last jmp */

#define  DMAT       3       /* value of matching bases */
#define  DMIS       0       /* penalty for mismatched bases */
#define  DINS0      8       /* penalty for a gap */
#define  DINS1      1       /* penalty per base */
#define  PINS0      8       /* penalty for a gap */
#define  PINS1      4       /* penalty per residue */

struct jmp {
        short           n[MAXJMP];      /* size of jmp (neg for dely) */
        unsigned short  x[MAXJMP];      /* base no. of jmp in seq x */
};                                      /* limits seq to 2^16 -1 */

struct diag {
        int             score;          /* score at last jmp */
        long            offset;         /* offset of prev block */
        short           ijmp;           /* current jmp index */
        struct jmp      jp;             /* list of jmps */
};

struct path {
        int     spc;            /* number of leading spaces */
        short   n[JMPS];/* size of jmp (gap) */
        int     x[JMPS];/* loc of jmp (last elem before gap) */
};

char            *ofile;                 /* output file name */
char            *namex[2];              /* seq names: getseqs() */
char            *prog;                  /* prog name for err msgs */
char            *seqx[2];               /* seqs: getseqs() */
int             dmax;                   /* best diag: nw() */
int             dmax0;                  /* final diag */
int             dna;                    /* set if dna: main() */
int             endgaps;                /* set if penalizing end gaps */
int             gapx, gapy;             /* total gaps in seqs */
int             len0, len1;             /* seq lens */
int             ngapx, ngapy;           /* total size of gaps */
int             smax;                   /* max score: nw() */
int             *xbm;                   /* bitmap for matching */
long            offset;                 /* current offset in jmp file */
struct  diag    *dx;                    /* holds diagonals */
struct  path    pp[2];                  /* holds path for seqs */

char            *calloc(), *malloc(), *index(), *strcpy();
char            *getseq(), *g_calloc();
```

Table 1 (cont')

```
/* Needleman-Wunsch alignment program
 *
 * usage: progs file1 file2
 *   where file1 and file2 are two dna or two protein sequences.
 *   The sequences can be in upper- or lower-case an may contain ambiguity
 *   Any lines beginning with ';', '>' or '<' are ignored
 *   Max file length is 65535 (limited by unsigned short x in the jmp struct)
 *   A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
 *   Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650
 */
#include "nw.h"
#include "day.h"

static    _dbval[26] = {
          1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static    _pbval[26] = {
          1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
          128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
          1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
          1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)
          main
          int       ac;
          char      *av[];
{
          prog = av[0];
          if (ac != 3) {
                    fprintf(stderr,"usage: %s file1 file2\n", prog);
                    fprintf(stderr,"where file1 and file2 are two dna or two protein sequences.\n");
                    fprintf(stderr,"The sequences can be in upper- or lower-case\n");
                    fprintf(stderr,"Any lines beginning with ';' or '<' are ignored\n");
                    fprintf(stderr,"Output is in the file \"align.out\"\n");
                    exit(1);
          }
          namex[0] = av[1];
          namex[1] = av[2];
          seqx[0] = getseq(namex[0], &len0);
          seqx[1] = getseq(namex[1], &len1);
          xbm = (dna)? _dbval : _pbval;

          endgaps = 0;                    /* 1 to penalize endgaps */
          ofile = "align.out";            /* output file */

          nw();             /* fill in the matrix, get the possible jmps */
          readjmps();       /* get the actual jmps */
          print();          /* print stats, alignment */

          cleanup(0);       /* unlink any tmp files */
}
```

Table 1 (cont')

```
/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
nw()                                                                    nw
{
        char            *px, *py;       /* seqs and ptrs */
        int             *ndely, *dely;  /* keep track of dely */
        int             ndelx, delx;    /* keep track of delx */
        int             *tmp;           /* for swapping row0, row1 */
        int             mis;            /* score for each type */
        int             ins0, ins1;     /* insertion penalties */
        register        id;             /* diagonal index */
        register        ij;             /* jmp index */
        register        *col0, *col1;   /* score for curr, last row */
        register        xx, yy;         /* index into seqs */

        dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

        ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
        dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
        col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
        col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
        ins0 = (dna)? DINS0 : PINS0;
        ins1 = (dna)? DINS1 : PINS1;

        smax = -10000;
        if (endgaps) {
                for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
                        col0[yy] = dely[yy] = col0[yy-1] - ins1;
                        ndely[yy] = yy;
                }
                col0[0] = 0;            /* Waterman Bull Math Biol 84 */
        }
        else
                for (yy = 1; yy <= len1; yy++)
                        dely[yy] = -ins0;

        /* fill in match matrix
         */
        for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
                /* initialize first entry in col
                 */
                if (endgaps) {
                        if (xx == 1)
                                col1[0] = delx = -(ins0+ins1);
                        else
                                col1[0] = delx = col0[0] - ins1;
                        ndelx = xx;
                }
                else {
                        col1[0] = 0;
                        delx = -ins0;
                        ndelx = 0;
                }
```

Table 1 (cont')

```
for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
        mis = col0[yy-1];
        if (dna)
                mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
        else
                mis += _day[*px-'A'][*py-'A'];

        /* update penalty for del in x seq;
         * favor new del over ongong del
         * ignore MAXGAP if weighting endgaps
         */
        if (endgaps || ndely[yy] < MAXGAP) {
                if (col0[yy] - ins0 >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else {
                        dely[yy] -= ins1;
                        ndely[yy]++;
                }
        } else {
                if (col0[yy] - (ins0+ins1) >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else
                        ndely[yy]++;
        }

        /* update penalty for del in y seq;
         * favor new del over ongong del
         */
        if (endgaps || ndelx < MAXGAP) {
                if (col1[yy-1] - ins0 >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else {
                        delx -= ins1;
                        ndelx++;
                }
        } else {
                if (col1[yy-1] - (ins0+ins1) >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else
                        ndelx++;
        }

        /* pick the maximum score; we're favoring
         * mis over any del and delx over dely
         */
```

Table 1 (cont')

```
                        id = xx - yy + len1 - 1;
        ...nw
                        if (mis >= delx && mis >= dely[yy])
                                col1[yy] = mis;
                        else if (delx >= dely[yy]) {
                                col1[yy] = delx;
                                ij = dx[id].ijmp;
                                if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
                                && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                                        dx[id].ijmp++;
                                        if (++ij >= MAXJMP) {
                                                writejmps(id);
                                                ij = dx[id].ijmp = 0;
                                                dx[id].offset = offset;
                                                offset += sizeof(struct jmp) + sizeof(offset);
                                        }
                                }
                                dx[id].jp.n[ij] = ndelx;
                                dx[id].jp.x[ij] = xx;
                                dx[id].score = delx;
                        }
                        else {
                                col1[yy] = dely[yy];
                                ij = dx[id].ijmp;
        if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
                                && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                                        dx[id].ijmp++;
                                        if (++ij >= MAXJMP) {
                                                writejmps(id);
                                                ij = dx[id].ijmp = 0;
                                                dx[id].offset = offset;
                                                offset += sizeof(struct jmp) + sizeof(offset);
                                        }
                                }
                                dx[id].jp.n[ij] = -ndely[yy];
                                dx[id].jp.x[ij] = xx;
                                dx[id].score = dely[yy];
                        }
                        if (xx == len0 && yy < len1) {
                                /* last col
                                 */
                                if (endgaps)
                                        col1[yy] -= ins0+ins1*(len1-yy);
                                if (col1[yy] > smax) {
                                        smax = col1[yy];
                                        dmax = id;
                                }
                        }
                }
                if (endgaps && xx < len0)
                        col1[yy-1] -= ins0+ins1*(len0-xx);
                if (col1[yy-1] > smax) {
                        smax = col1[yy-1];
                        dmax = id;
                }
                tmp = col0; col0 = col1; col1 = tmp;
        }
        (void) free((char *)ndely);
        (void) free((char *)dely);
        (void) free((char *)col0);
        (void) free((char *)col1);
}
```

nw.c

Table 1 (cont')

```
/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() - -put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC     3
#define P_LINE  256     /* maximum output line */
#define P_SPC   3       /* space between name or num and seq */

extern  _day[26][26];
int     olen;           /* set output line length */
FILE    *fx;            /* output file */

print()
        print
{
        int     lx, ly, firstgap, lastgap;      /* overlap */

        if ((fx = fopen(ofile, "w")) == 0) {
                fprintf(stderr, "%s: can't write %s\n", prog, ofile);
                cleanup(1);
        }
        fprintf(fx, "< first sequence: %s (length = %d)\n", namex[0], len0);
        fprintf(fx, "< second sequence: %s (length = %d)\n", namex[1], len1);
        olen = 60;
        lx = len0;
        ly = len1;
        firstgap = lastgap = 0;
        if (dmax < len1 - 1) {          /* leading gap in x */
                pp[0].spc = firstgap = len1 - dmax - 1;
                ly -= pp[0].spc;
        }
        else if (dmax > len1 - 1) {     /* leading gap in y */
                pp[1].spc = firstgap = dmax - (len1 - 1);
                lx -= pp[1].spc;
        }
        if (dmax0 < len0 - 1) {         /* trailing gap in x */
                lastgap = len0 - dmax0 -1;
                lx -= lastgap;
        }
        else if (dmax0 > len0 - 1) {    /* trailing gap in y */
                lastgap = dmax0 - (len0 - 1);
                ly -= lastgap;
        }
        getmat(lx, ly, firstgap, lastgap);
        pr_align();
}
```

Table 1 (cont')

```
/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)                                    getmat
        int     lx, ly;                 /* "core" (minus endgaps) */
        int     firstgap, lastgap;      /* leading trailing overlap */
{
        int             nm, i0, i1, siz0, siz1;
        char            outx[32];
        double          pct;
        register        n0, n1;
        register char   *p0, *p1;

        /* get total matches, score
         */
        i0 = i1 = siz0 = siz1 = 0;
        p0 = seqx[0] + pp[1].spc;
        p1 = seqx[1] + pp[0].spc;
        n0 = pp[1].spc + 1;
        n1 = pp[0].spc + 1;

        nm = 0;
        while ( *p0 && *p1 ) {
                if (siz0) {
                        p1++;
                        n1++;
                        siz0--;
                }
                else if (siz1) {
                        p0++;
                        n0++;
                        siz1--;
                }
                else {
                        if (xbm[*p0-'A']&xbm[*p1-'A'])
                                nm++;
                        if (n0++ == pp[0].x[i0])
                                siz0 = pp[0].n[i0++];
                        if (n1++ == pp[1].x[i1])
                                siz1 = pp[1].n[i1++];
                        p0++;
                        p1++;
                }
        }

        /* pct homology:
         * if penalizing endgaps, base is the shorter seq
         * else, knock off overhangs and take shorter core
         */
        if (endgaps)
                lx = (len0 < len1)? len0 : len1;
        else
                lx = (lx < ly)? lx : ly;
        pct = 100.*(double)nm/(double)lx;
        fprintf(fx, "\n");
        fprintf(fx, " < %d match%s in an overlap of %d: %.2f percent similarity\n",
                nm, (nm == 1)? "" : "es", lx, pct);
```

Table 1 (cont')

...getmat

```
        fprintf(fx, "< gaps in first sequence: %d", gapx);
        if (gapx) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapx, (dna)? "base":"residue", (ngapx == 1)? "":"s");
                fprintf(fx,"%s", outx);

        fprintf(fx, ", gaps in second sequence: %d", gapy);
        if (gapy) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapy, (dna)? "base":"residue", (ngapy == 1)? "":"s");
                fprintf(fx,"%s", outx);
        }
        if (dna)
                fprintf(fx,
                "\n< score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
                smax, DMAT, DMIS, DINS0, DINS1);
        else
                fprintf(fx,
                "\n< score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
                smax, PINS0, PINS1);
        if (endgaps)
                fprintf(fx,
                "< endgaps penalized, left endgap: %d %s%s, right endgap: %d %s%s\n",
                firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
                lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
        else
                fprintf(fx, "< endgaps not penalized\n");
}

static          nm;             /* matches in core -- for checking */
static          lmax;           /* lengths of stripped file names */
static          ij[2];          /* jmp index for a path */
static          nc[2];          /* number at start of current line */
static          ni[2];          /* current elem number -- for gapping */
static          siz[2];
static char     *ps[2];         /* ptr to current element */
static char     *po[2];         /* ptr to next output char slot */
static char     out[2][P_LINE]; /* output line */
static char     star[P_LINE];   /* set by stars() */

/*
 * print alignment of described in struct path pp[]
 */
static                                                          pr_align
pr_align()
{
        int             nn;     /* char count */
        int             more;
        register        i;

        for (i = 0, lmax = 0; i < 2; i++) {
                nn = stripname(namex[i]);
                if (nn > lmax)
                        lmax = nn;

                nc[i] = 1;
                ni[i] = 1;
                siz[i] = ij[i] = 0;
                ps[i] = seqx[i];
                po[i] = out[i];
        }
```

Table 1 (cont')

```
        for (nn = nm = 0, more = 1; more; ) {                                   ...pr_align
                for (i = more = 0; i < 2; i++) {
                        /*
                         * do we have more of this sequence?
                         */
                        if (!*ps[i])
                                continue;

                        more++;

                        if (pp[i].spc) {        /* leading space */
                                *po[i]++ = ' ';
                                pp[i].spc--;
                        }
                        else if (siz[i]) {      /* in a gap */
                                *po[i]++ = '-';
                                siz[i]--;
                        }
                        else {                  /* we're putting a seq element
                                                 */
                                *po[i] = *ps[i];
                                if (islower(*ps[i]))
                                        *ps[i] = toupper(*ps[i]);
                                po[i]++;
                                ps[i]++;

                                /*
                                 * are we at next gap for this seq?
                                 */
                                if (ni[i] == pp[i].x[ij[i]]) {
                                        /*
                                         * we need to merge all gaps
                                         * at this location
                                         */
                                        siz[i] = pp[i].n[ij[i]++];
                                        while (ni[i] == pp[i].x[ij[i]])
                                                siz[i] += pp[i].n[ij[i]++];
                                }
                                ni[i]++;
                        }
                }
                if (++nn == olen || !more && nn) {
                        dumpblock();
                        for (i = 0; i < 2; i++)
                                po[i] = out[i];
                        nn = 0;
                }
        }
}

/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
static
dumpblock()                                                                     dumpblock
{
        register  i;

        for (i = 0; i < 2; i++)
                *po[i]-- = '\0';
```

Table 1 (cont')

...dumpblock

```
        (void) putc('\n', fx);
        for (i = 0; i < 2; i++) {
                if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
                        if (i == 0)
                                nums(i);
                        if (i == 0 && *out[1])
                                stars();
                        putline(i);
                        if (i == 0 && *out[1])
                                fprintf(fx, star);
                        if (i == 1)
                                nums(i);
                }
        }
}

/*
 * put out a number line: dumpblock()
 */
static
nums(ix)                                                        nums
        int     ix;     /* index in out[] holding seq line */
{
        char            nline[P_LINE];
        register        i, j;
        register char   *pn, *px, *py;

        for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
                *pn = ' ';
        for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
                if (*py == ' ' || *py == '-')
                        *pn = ' ';
                else {
                        if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                                j = (i < 0)? -i : i;
                                for (px = pn; j; j /= 10, px--)
                                        *px = j%10 + '0';
                                if (i < 0)
                                        *px = '-';
                        }
                        else
                                *pn = ' ';
                        i++;
                }
        }
        *pn = '\0';
        nc[ix] = i;
        for (pn = nline; *pn; pn++)
                (void) putc(*pn, fx);
        (void) putc('\n', fx);
}

/*
 * put out a line (name, [num], seq, [num]): dumpblock()
 */
static
putline(ix)                                                     putline
        int     ix;
{
```

Table 1 (cont')

...putline

```
        int             i;
        register char   *px;

        for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
                (void) putc(*px, fx);
        for (; i < lmax+P_SPC; i++)
                (void) putc(' ', fx);

        /* these count from 1:
         * ni[] is current element (from 1)
         * nc[] is number at start of current line
         */
        for (px = out[ix]; *px; px++)
                (void) putc(*px&0x7F, fx);
        (void) putc('\n', fx);
}

/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock()
 */
static
stars()
        stars
{
        int             i;
        register char   *p0, *p1, cx, *px;

        if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ' ') ||
           !*out[1] || (*out[1] == ' ' && *(po[1]) == ' '))
                return;
        px = star;
        for (i = lmax+P_SPC; i; i--)
                *px++ = ' ';

        for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
                if (isalpha(*p0) && isalpha(*p1)) {

                        if (xbm[*p0-'A']&xbm[*p1-'A']) {
                                cx = '*';
                                nm++;
                        }
                        else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                                cx = '.';
                        else
                                cx = ' ';
                }
                else
                        cx = ' ';
                *px++ = cx;
        }
        *px++ = '\n';
        *px = '\0';
}
```

Table 1 (cont')

```
/*
 * strip path or prefix from pn, return len: pr_align()
 */
static                                                          stripname
stripname(pn)
        char    *pn;    /* file name (may be path) */
{
        register char   *px, *py;

        py = 0;
        for (px = pn; *px; px++)
                if (*px == '/')
                        py = px + 1;
        if (py)
                (void) strcpy(pn, py);
        return(strlen(pn));

}
```

Table 1 (cont')

```
/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_calloc() -- calloc() with error checkin
 * readjmps() -- get the good jmps, from tmp file if necessary
 * writejmps() -- write a filled array of jmps to a tmp file: nwt)
 */
#include "nw.h"
#include <sys/file.h>

char    *jname = "/tmp/homgXXXXXX";             /* tmp file for jmps */
FILE    *fj;

int     cleanup();                              /* cleanup tmp file */
long    lseek();

/*
 * remove any tmp file if we blow
 */
cleanup(i)                                                              cleanup
        int     i;
{
        if (fj)
                (void) unlink(jname);
        exit(i);
}

/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ':', '<', or '>'
 * seq in upper or lower case
 */
char    *
getseq(file, len)                                                       getseq
        char    *file;  /* file name */
        int     *len;   /* seq len */
{
        char            line[1024], *pseq;
        register char   *px, *py;
        int             natgc, tlen;
        FILE            *fp;

        if ((fp = fopen(file,"r")) == 0) {
                fprintf(stderr,"%s: can't read %s\n", prog, file);
                exit(1);
        }
        tlen = natgc = 0;
        while (fgets(line, 1024, fp)) {
                if (*line == ':' || *line == '<' || *line == '>')
                        continue;
                for (px = line; *px != '\n'; px++)
                        if (isupper(*px) || islower(*px))
                                tlen++;
        }
        if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
                fprintf(stderr,"%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
                exit(1);
        }
        pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
```

Table 1 (cont')

...getseq

```
        py = pseq + 4;
        *len = tlen;
        rewind(fp);

        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '<' || *line == '>')
                        continue;
                for (px = line; *px != '\n'; px++) {
                        if (isupper(*px))
                                *py++ = *px;
                        else if (islower(*px))
                                *py++ = toupper(*px);
                        if (index("ATGCU",*(py-1)))
                                natgc++;
                }
        }
        *py++ = '\0';
        *py = '\0';
        (void) fclose(fp);
        dna = natgc > (tlen/3);
        return(pseq+4);
}

char    *
g_calloc(msg, nx, sz)                                                   g_calloc
        char    *msg;           /* program, calling routine */
        int     nx, sz;         /* number and size of elements */
{
        char            *px, *calloc();

        if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
                if (*msg) {
                        fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
                        exit(1);
                }
        }
        return(px);
}

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */
readjmps()                                                              readjmps
{
        int             fd = -1;
        int             siz, i0, i1;
        register        i, j, xx;

        if (fj) {
                (void) fclose(fj);
                if ((fd = open(jname, O_RDONLY, 0)) < 0) {
                        fprintf(stderr, "%s: can't open() %s\n", prog, jname);
                        cleanup(1);
                }
        }
        for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
                while (1) {
                        for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                                ;
```

Table 1 (cont')

...readjmps

```
                if (j < 0 && dx[dmax].offset && fj) {
                        (void) lseek(fd, dx[dmax].offset, 0);
                        (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
                        (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
                        dx[dmax].ijmp = MAXJMP-1;
                }
                else
                        break;
        }
        if (i >= JMPS) {
                fprintf(stderr, "%s: too many gaps in alignment\n", prog);
                cleanup(1);
        }
        if (j >= 0) {
                siz = dx[dmax].jp.n[j];
                xx = dx[dmax].jp.x[j];
                dmax += siz;
                if (siz < 0) {                  /* gap in second seq */
                        pp[1].n[i1] = -siz;
                        xx += siz;
                        /* id = xx - yy + len1 - 1
                         */
                        pp[1].x[i1] = xx - dmax + len1 - 1;
                        gapy++;
                        ngapy -= siz;
/* ignore MAXGAP when doing endgaps */
                        siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
                        i1++;
                }
                else if (siz > 0) {  /* gap in first seq */
                        pp[0].n[i0] = siz;
                        pp[0].x[i0] = xx;
                        gapx++;
                        ngapx += siz;
/* ignore MAXGAP when doing endgaps */
                        siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
                        i0++;
                }
        }
        else
                break;
    }
    /* reverse the order of jmps
     */
    for (j = 0, i0--; j < i0; j++, i0--) {
            i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
            i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
    }
    for (j = 0, i1--; j < i1; j++, i1--) {
            i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
            i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
    }
    if (fd >= 0)
            (void) close(fd);
    if (fj) {
            (void) unlink(jname);
            fj = 0;
            offset = 0;
    }
}
```

Table 1 (cont')

```
/*
 * write a filled jmp struct offset of the prev one (if any): nw()
 */
writejmps(ix)       writejmps
        int         ix;
{
        char        *mktemp();

        if (!fj) {
                if (mktemp(jname) < 0) {
                        fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
                        cleanup(1);
                }
                if ((fj = fopen(jname, "w")) == 0) {
                        fprintf(stderr, "%s: can't write %s\n", prog, jname);
                        exit(1);
                }
        }
        (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
        (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}
```

Table 2

PRO	XXXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXYYYYYYY	(Length = 12 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 15 = 33.3%

Table 3

PRO	XXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXYYYYYYZZYZ	(Length = 15 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 10 = 50%

Table 4

PRO-DNA	NNNNNNNNNNNNN	(Length = 14 nucleotides)
Comparison DNA	NNNNNNLLLLLLLLL	(Length = 16 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

6 divided by 14 = 42.9%

Table 5

PRO-DNA	NNNNNNNNNNNN	(Length = 12 nucleotides)
Comparison DNA	NNNNLLLVV	(Length = 9 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

4 divided by 12 = 33.3%

II. Compositions and Methods of the Invention

1. Preparation of the PRO polypeptides of the invention

The present invention provides newly identified and isolated nucleotide sequences encoding the polypeptides in the present application as PRO polypeptides. In particular, cDNAs encoding various PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separete expression rounds may be given different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for the sake of simplicity, in the present specification the protein encoded by the full length native nucleic acid molecules disclosed herein as well as all further native homologues and variants included in the foregoing definition of PRO, will be referred to as "PRO/number" or even "PRO", regardless of their origin or mode of preparation.

In particular, cDNA encoding a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 and PRO5727 polypeptide (corresponding to UNQ174, UNQ178, UNQ186, UNQ190, UNQ200, UNQ214, UNQ209, UNQ219, UNQ146, UNQ240, UNQ239, UNQ293, UNQ330, UNQ365, UNQ316, UNQ317, UNQ318, UNQ319, UNQ313, UNQ332, UNQ334, UNQ540, UNQ434, UNQ408, UNQ407, UNQ430, UNQ557, UNQ491, UNQ598, UNQ516, UNQ701, UNQ585, UNQ633, UNQ678, UNQ606, UNQ630, UNQ653, UNQ608, UNQ698, UNQ732, UNQ722, UNQ728, UNQ900, UNQ859, UNQ179, UNQ21, UNQ236, UNQ303, UNQ294, UNQ322, UNQ388, UNQ435, UNQ433, UNQ483, UNQ545, UNQ589, UNQ707, UNQ685, UNQ693, UNQ739, UNQ1888, UNQ1866, UNQ1947 and UNQ2448, respectively) has been identified and isolated, as disclosed in further detail in the Examples below.

In even greater particularity, the present specification describes the cDNAs DNA29101-1276, DNA30871-1157, DNA30942-1134, DNA33087-1158, DNA33460-1166, DNA34387-1138, DNA35558-1167, DNA35638-1141, DNA35916-1161, DNA39523-1192, DNA40620-1183, DNA40982-1235, DNA44184-1319, DNA44205-1285, DNA45410-1250, DNA45416-1251, DNA45419-1252, DNA47365-1206, DNA47470-1130, DNA48314-1320, DNA49435-1219, DNA50921-1458, DNA53974-1401, DNA54228-1366, DNA54231-1366, DNA56405-1357, DNA57033-1403, DNA57690-1374, DNA59220-1514, DNA59294-1381, DNA59776-1600, DNA59849-1504, DNA60775-1532, DNA61873-1574, DNA62814-1521, DNA64885-1529, DNA65404-1551, DNA65412-1523, DNA66675-1587, DNA68864-1629, DNA68872-1620, DNA68874-1622, DNA76400-2528, DNA77624-2515, DNA30868-1156, DNA36638-1056, DNA38260-1180, DNA40592-1242, DNA41374-1312, DNA44194-1317, DNA53517-1366, DNA53971-1359, DNA53987-1438, DNA57700-1408, DNA59620-1463, DNA60627-1508, DNA64890-1612, DNA66659-1593, DNA66667-1596, DNA68818-2536, DNA84210-2576, DNA92218-2554, DNA96878-2626, DNA98853-1739 which encode native sequence PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769,

PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 and PRO5727 polypeptides, respectively.

As disclosed in the Examples below, various cDNA clones have been deposited with the ATCC. The actual nucleotide sequence of those clones can readily be determined by the skilled artisan by sequencing of the deposited clone using routine methods in the art. It is understood that the sequence of the deposit contains the correct sequence in the event of a discrepancy between the deposited sequence and those disclosed herein. The predicted amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO polypeptides and encoding nucleic acids described herein, Applicants have identified what is believed to be the reading frame best identifiable with the sequence information available at the time.

B. PRO Polypeptide Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length PRO sequence or in various domains of the PRO described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO that results in a change in the amino acid sequence of the PRO as compared with the native sequence PRO. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

PRO polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO polypeptide.

PRO fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO fragments by enzymatic digestion, *e.g.*, by treating the protein with an enzyme known to cleave proteins at sites defined by

particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO polypeptide fragments share at least one biological and/or immunological activity with the native PRO polypeptide disclosed herein.

In particular embodiments, conservative substitutions of interest are shown in Table 6 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 6, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 6

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro; ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe;	
	norleucine	leu
Leu (L)	norleucine; ile; val;	
	met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	leu
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe;	
	ala; norleucine	leu

Substantial modifications in function or immunological identity of the invention polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure

of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter *et al.*, *Nucl. Acids Res.*, 13:4331 (1986); Zoller *et al.*, *Nucl. Acids Res.*, 10:6487 (1987)], cassette mutagenesis [Wells *et al.*, *Gene*, 34:315 (1985)], restriction selection mutagenesis [Wells *et al.*, *Philos. Trans. R. Soc. London SerA*, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, *Science*, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

C. Modifications of PRO

Covalent modifications of PRO polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate.

Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side

chains [T.E. Creighton. *Proteins: Structure and Molecular Properties*. W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO polypeptide (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO (for O-linked glycosylation sites). The PRO amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, *e.g.*, in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.*, 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.*, 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.*, 138:350 (1987).

Another type of covalent modification of PRO comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO polypeptides may also be modified in a way to form a chimeric molecule comprising the invention polypeptide fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO. The presence of such epitope-tagged forms of the PRO polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.*, *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc

tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp *et al.*, *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin *et al.*, *Science*, 255:192-194 (1992)]; an α-tubulin epitope peptide [Skinner *et al.*, *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of an invention polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

D. Preparation of PRO

The description below relates to primarily to production of PRO by culturing cells transformed or transfected with a vector containing PRO nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO. For instance, the PRO sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.* 85: 2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using the manufacturer's instructions. Various portions of the PRO may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO.

1. Isolation of DNA Encoding the PRO Polypeptide(s)

DNA encoding the PRO may be obtained from a cDNA library prepared from tissue believed to possess the polypeptide mRNA and to express it at a detectable level. Accordingly, human PRO DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO-encoding gene may also be obtained from a genomic library, oligonucleotide synthesis, or other known synthetic procedures (*e.g.*, automated nucleic acid synthesis).

Libraries can be screened with probes (such as antibodies to the PRO polypeptide or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding the PRO polypeptide is to use PCR methodology [Sambrook *et al.*, *supra*; Dieffenbach *et al.*, *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives

are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ^{32}P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al., supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al., supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for production of the PRO polypeptides and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al., supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al., supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al., Gene*, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology*, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al., J. Bact.*, 130:946 (1977) and *Hsiao et al., Proc. Natl. Acad. Sci. (USA)*, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al., Methods in Enzymology*, 185:527-537 (1990) and Mansour *et al., Nature*, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia, e.g., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella,*

e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans, and *Shigella,* as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g., B. licheniformis* 41P disclosed in DD 266.710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa,* and *Streptomyces.* These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA ; E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3; E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan*^{r}; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan*^{r}; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, *e.g.,* PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature,* 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al., Bio/Technology,* 9:968-975 (1991)) such as, *e.g., K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al., J. Bacteriol.,* 154(2):737-742 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906; Van den Berg *et al., Bio/Technology,* 8:135 (1990)), *K. thermotolerans,* and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al., J. Basic Microbiol.,* 28:265-278 [1988]); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al., Proc. Natl. Acad. Sci. USA,* 76:5259-5263 [1979]); Schwanniomyces such as Schwanniomyces *occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, *e.g., Neurospora, Penicillium, Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al., Biochem. Biophys. Res. Commun.,* 112:284-289 [1983]; Tilburn *et al., Gene,* 26:205-221 [1983]; Yelton *et al., Proc. Natl. Acad. Sci. USA,* 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, *EMBO J.,* 4:475-479 [1985]). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula, Candida, Kloeckera, Pichia, Saccharomyces, Torulopsis,* and *Rhodotorula.* A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylotrophs,* 269 (1982).

Suitable host cells for the expression of glycosylated PRO polypeptides are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as Drosophila S2 and Spodoptera Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al., J. Gen Virol.* 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse

mammary tumor (MMT 060562. ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

3. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding the PRO polypeptides may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, phagemid or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 [Stinchcomb *et al.*, *Nature*, 282:39 (1979); Kingsman *et al.*, *Gene*, 7:141 (1979); Tschemper *et al.*, *Gene*, 10:157 (1980)]. The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example,

ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems [Chang *et al.*, *Nature*, 275:615 (1978); Goeddel *et al.*, *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman *et al.*, *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess *et al.*, *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phospho-fructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding the PRO polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the coding sequence of the PRO polypeptide, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of the PRO polypeptide in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

4. Detecting Gene Expression

Gene expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to DNA encoding the PRO polypeptide and encoding a specific antibody epitope.

5. Purification of Polypeptide

Forms of the PRO may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.*, Triton™-X 100) or by enzymatic cleavage. Cells employed in expression of the PRO polypeptide can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO polypeptide from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO polypeptide. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, *Methods in Enzymology*, 182 (1990); Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO polypeptide produced.

E. Tissue Distribution

The location of tissues expressing the PRO can be identified by determining mRNA expression in various human tissues. The location of such genes provides information about which tissues are most likely to be affected by the stimulating and inhibiting activities of the PRO polypeptides. The location of a gene in a specific tissue also provides sample tissue for the activity blocking assays discussed below.

As noted before, gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence of a PRO polypeptide or against a synthetic peptide based on the DNA sequences encoding the PRO polypeptide or against an exogenous sequence fused to a DNA encoding a PRO polypeptide and encoding a specific antibody epitope. General techniques for generating antibodies, and special protocols for Northern blotting and *in situ* hybridization are provided below.

F. Antibody Binding Studies

The activity of the PRO polypeptides can be further verified by antibody binding studies, in which the ability of anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibodies to inhibit the effect of the PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptides, respectively, on tissue cells is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate

determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, *e.g.*, US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

G. Cell-Based Assays

Cell-based assays and animal models for immune related diseases can be used to further understand the relationship between the genes and polypeptides identified herein and the development and pathogenesis of immune related disease.

In a different approach, cells of a cell type known to be involved in a particular immune related disease are transfected with the cDNAs described herein, and the ability of these cDNAs to stimulate or inhibit immune function is analyzed. Suitable cells can be transfected with the desired gene, and monitored for immune function activity. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit or stimulate immune function, for example to modulate T-cell proliferation or inflammatory cell infiltration. Cells transfected with the coding sequences of the genes identified herein can further be used to identify drug candidates for the treatment of immune related diseases.

In addition, primary cultures derived from transgenic animals (as described below) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous cell lines from transgenic animals are well known in the art (see, *e.g.*, Small *et al.*, *Mol. Cell. Biol.* 5: 642-648 [1985]).

One suitable cell based assay is the mixed lymphocyte reaction (MLR). *Current Protocols in Immunology*, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Institutes of Health, Published by John Wiley & Sons, Inc. In this assay, the ability of a test compound to stimulate or inhibit the proliferation of activated T cells is assayed. A suspension of responder T cells is cultured with allogeneic stimulator cells and the proliferation of T cells is measured by uptake of tritiated thymidine. This assay is a general measure of T cell reactivity. Since the majority of T cells respond to and produce IL-2 upon activation, differences in responsiveness in this assay in part reflect differences in IL-2 production by the responding cells. The MLR results can be verified by a standard lymphokine (IL-2) detection assay. *Current Protocols in Immunology*, above, 3.15, 6.3.

A proliferative T cell response in an MLR assay may be due to direct mitogenic properties of an assayed molecule or to external antigen induced activation. Additional verification of the T cell stimulatory activity of the PRO polypeptides can be obtained by a costimulation assay. T cell activation requires an antigen specific signal mediated through the T-cell receptor (TCR) and a costimulatory signal mediated

through a second ligand binding interaction, for example, the B7 (CD80, CD86)/CD28 binding interaction. CD28 crosslinking increases lymphokine secretion by activated T cells. T cell activation has both negative and positive controls through the binding of ligands which have a negative or positive effect. CD28 and CTLA-4 are related glycoproteins in the Ig superfamily which bind to B7. CD28 binding to B7 has a positive costimulation effect of T cell activation; conversely, CTLA-4 binding to B7 has a negative T cell deactivating effect. Chambers, C. A. and Allison, *J. P., Curr. Opin. Immunol.* (1997) 9:396. Schwartz, R. H., *Cell* (1992) 71:1065; Linsey, P. S. and Ledbetter, J. A., *Annu. Rev. Immunol.* (1993) 11:191; June, C. H. *et al. Immunol. Today* (1994) 15:321; Jenkins, M. K., *Immunity* (1994) 1:405. In a costimulation assay, the PRO polypeptides are assayed for T cell costimulatory or inhibitory activity.

PRO polypeptides, as well as other compounds of the invention, which are stimulators (costimulators) of T cell proliferation and agonists, *e.g.*, agonist antibodies, thereto as determined by MLR and costimulation assays, for example, are useful in treating immune related diseases characterized by poor, suboptimal or inadequate immune function. These diseases are treated by stimulating the proliferation and activation of T cells (and T cell mediated immunity) and enhancing the immune response in a mammal through administration of a stimulatory compound, such as the stimulating PRO polypeptides. The stimulating polypeptide may, for example, be a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide or an agonist antibody thereof.

Direct use of a stimulating compound as in the invention has been validated in experiments with 4-1BB glycoprotein, a member of the tumor necrosis factor receptor family, which binds to a ligand (4-1BBL) expressed on primed T cells and signals T cell activation and growth. Alderson, M. E. *et al., J. Immunol.* (1994) 24:2219.

The use of an agonist stimulating compound has also been validated experimentally. Activation of 4-1BB by treatment with an agonist anti-4-1BB antibody enhances eradication of tumors. Hellstrom, I. and Hellstrom, K. E., *Crit. Rev. Immunol.* (1998) 18:1. Immunoadjuvant therapy for treatment of tumors, described in more detail below, is another example of the use of the stimulating compounds of the invention.

An immune stimulating or enhancing effect can also be achieved by antagonizing or blocking the activity of a PRO which has been found to be inhibiting in the MLR assay. Negating the inhibitory activity of the compound produces a net stimulatory effect. Suitable antagonists/blocking compounds are antibodies or fragments thereof which recognize and bind to the inhibitory protein, thereby blocking the effective interaction of the protein with its receptor and inhibiting signaling through the receptor. This effect has been validated in experiments using anti-CTLA-4 antibodies which enhance T cell proliferation, presumably by removal of the inhibitory signal caused by CTLA-4 binding. Walunas, T. L. *et al. Immunity* (1994) 1:405.

Alternatively, an immune stimulating or enhancing effect can also be achieved by administration of a PRO which has vascular permeability enhancing properties. Enhanced vacuolar permeability would be

beneficial to disorders which can be attenuated by local infiltration of immune cells (*e.g.*, monocytes, eosinophils, PMNs) and inflammation.

On the other hand, PRO polypeptides, as well as other compounds of the invention, which are direct inhibitors of T cell proliferation/activation, lymphokine secretion, and/or vascular permeability can be directly used to suppress the immune response. These compounds are useful to reduce the degree of the immune response and to treat immune related diseases characterized by a hyperactive, superoptimal, or autoimmune response. This use of the compounds of the invention has been validated by the experiments described above in which CTLA-4 binding to receptor B7 deactivates T cells. The direct inhibitory compounds of the invention function in an analogous manner. The use of compound which suppress vascular permeability would be expected to reduce inflammation. Such uses would be beneficial in treating conditions associated with excessive inflammation.

Alternatively, compounds, *e.g.*, antibodies, which bind to stimulating PRO polypeptides and block the stimulating effect of these molecules produce a net inhibitory effect and can be used to suppress the T cell mediated immune response by inhibiting T cell proliferation/activation and/or lymphokine secretion. Blocking the stimulating effect of the polypeptides suppresses the immune response of the mammal. This use has been validated in experiments using an anti-IL2 antibody. In these experiments, the antibody binds to IL2 and blocks binding of IL2 to its receptor thereby achieving a T cell inhibitory effect.

H. Animal Models

The results of the cell based in vitro assays can be further verified using *in vivo* animal models and assays for T-cell function. A variety of well known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of immune related disease, and to test the efficacy of candidate therapeutic agents, including antibodies, and other antagonists of the native polypeptides, including small molecule antagonists. The *in vivo* nature of such models makes them predictive of responses in human patients. Animal models of immune related diseases include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, *e.g.*, murine models. Such models can be generated by introducing cells into syngeneic mice using standard techniques, *e.g.*, subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, *etc.*

Graft-versus-host disease occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor cells recognize and respond to host antigens. The response can vary from life threatening severe inflammation to mild cases of diarrhea and weight loss. Graft-versus-host disease models provide a means of assessing T cell reactivity against MHC antigens and minor transplant antigens. A suitable procedure is described in detail in Current Protocols in Immunology, above, unit 4.3.

An animal model for skin allograft rejection is a means of testing the ability of T cells to mediate *in vivo* tissue destruction and a measure of their role in transplant rejection. The most common and accepted models use murine tail-skin grafts. Repeated experiments have shown that skin allograft rejection is mediated by T cells, helper T cells and killer-effector T cells, and not antibodies. Auchincloss, H. Jr. and Sachs, D. H., *Fundamental Immunology*, 2nd ed., W. E. Paul ed., Raven Press, NY, 1989, 889-992. A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.4. Other transplant rejection models

which can be used to test the compounds of the invention are the allogeneic heart transplant models described by Tanabe, M. *et al. Transplantation* (1994) 58:23 and Tinubu, S. A. *et al. J. Immunol.* (1994) 4330-4338.

Animal models for delayed type hypersensitivity provides an assay of cell mediated immune function as well. Delayed type hypersensitivity reactions are a T cell mediated *in vivo* immune response characterized by inflammation which does not reach a peak until after a period of time has elapsed after challenge with an antigen. These reactions also occur in tissue specific autoimmune diseases such as multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE, a model for MS). A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.5.

EAE is a T cell mediated autoimmune disease characterized by T cell and mononuclear cell inflammation and subsequent demyelination of axons in the central nervous system. EAE is generally considered to be a relevant animal model for MS in humans. Bolton, C., *Multiple Sclerosis* (1995) 1:143. Both acute and relapsing-remitting models have been developed. The compounds of the invention can be tested for T cell stimulatory or inhibitory activity against immune mediated demyelinating disease using the protocol described in *Current Protocols in Immunology*, above, units 15.1 and 15.2. See also the models for myelin disease in which oligodendrocytes or Schwann cells are grafted into the central nervous system as described in Duncan, I. D. *et al. Molec. Med. Today* (1997) 554-561.

Contact hypersensitivity is a simple delayed type hypersensitivity *in vivo* assay of cell mediated immune function. In this procedure, cutaneous exposure to exogenous haptens which gives rise to a delayed type hypersensitivity reaction which is measured and quantitated. Contact sensitivity involves an initial sensitizing phase followed by an elicitation phase. The elicitation phase occurs when the T lymphocytes encounter an antigen to which they have had previous contact. Swelling and inflammation occur, making this an excellent model of human allergic contact dermatitis. A suitable procedure is described in detail in *Current Protocols in Immunology*, Eds. J. E. Cologan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, John Wiley & Sons, Inc., 1994, unit 4.2. See also Grabbe, S. and Schwarz, T, *Immun. Today* 19 (1): 37-44 (1998).

An animal model for arthritis is collagen-induced arthritis. This model shares clinical, histological and immunological characteristics of human autoimmune rheumatoid arthritis and is an acceptable model for human autoimmune arthritis. Mouse and rat models are characterized by synovitis, erosion of cartilage and subchondral bone. The compounds of the invention can be tested for activity against autoimmune arthritis using the protocols described in *Current Protocols in Immunology*, above, units 15.5. See also the model using a monoclonal antibody to CD18 and VLA-4 integrins described in Issekutz, A.C. *et al., Immunology* (1996) 88:569.

A model of asthma has been described in which antigen-induced airway hyper-reactivity, pulmonary eosinophilia and inflammation are induced by sensitizing an animal with ovalbumin and then challenging the animal with the same protein delivered by aerosol. Several animal models (guinea pig, rat, non-human primate) show symptoms similar to atopic asthma in humans upon challenge with aerosol antigens. Murine models have many of the features of human asthma. Suitable procedures to test the compounds of the invention for activity and effectiveness in the treatment of asthma are described by Wolyniec, W. W. *et al, Am. J. Respir. Cell Mol. Biol.* (1998) 18:777 and the references cited therein.

Additionally, the compounds of the invention can be tested on animal models for psoriasis like diseases. Evidence suggests a T cell pathogenesis for psoriasis. The compounds of the invention can be tested in the scid/scid mouse model described by Schon, M. P. *et al, Nat. Med.* (1997) 3:183, in which the mice demonstrate histopathologic skin lesions resembling psoriasis. Another suitable model is the human skin/scid mouse chimera prepared as described by Nickoloff, B. J. *et al, Am. J. Path.* (1995) 146:580.

Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the genes identified herein into the genome of animals of interest, using standard techniques for producing transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, *e.g.*, baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ lines (*e.g.*, Van der Putten *et al., Proc. Natl. Acad. Sci. USA* 82, 6148-615 [1985]); gene targeting in embryonic stem cells (Thompson *et al., Cell* 56, 313-321 [1989]); electroporation of embryos (Lo, *Mol. Cel.. Biol.* 3, 1803-1814 [1983]); sperm-mediated gene transfer (Lavitrano *et al., Cell* 57, 717-73 [1989]). For review, see, for example, U.S. Patent No. 4,736,866.

For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers, *e.g.*, head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko *et al., Proc. Natl. Acad. Sci. USA* 89, 6232-636 (1992).

The expression of the transgene in transgenic animals can be monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR, or immunocytochemistry.

The animals may be further examined for signs of immune disease pathology, for example by histological examination to determine infiltration of immune cells into specific tissues. Blocking experiments can also be performed in which the transgenic animals are treated with the compounds of the invention to determine the extent of the T cell proliferation stimulation or inhibition of the compounds. In these experiments, blocking antibodies which bind to the PRO polypeptide, prepared as described above, are administered to the animal and the effect on immune function is determined.

Alternatively, "knock out" animals can be constructed which have a defective or altered gene encoding a polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a particular polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see *e.g.*, Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced DNA has homologously

recombined with the endogenous DNA are selected [see *e.g.*, Li *et al.*, *Cell*, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse or rat) to form aggregation chimeras [see *e.g.*, Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the polypeptide.

I. ImmunoAdjuvant Therapy

In one embodiment, the immunostimulating compounds of the invention can be used in immunoadjuvant therapy for the treatment of tumors (cancer). It is now well established that T cells recognize human tumor specific antigens. One group of tumor antigens, encoded by the MAGE, BAGE and GAGE families of genes, are silent in all adult normal tissues , but are expressed in significant amounts in tumors, such as melanomas, lung tumors, head and neck tumors, and bladder carcinomas. DeSmet, C. *et al.*, (1996) *Proc. Natl. Acad. Sci. USA*, 93:7149. It has been shown that costimulation of T cells induces tumor regression and an antitumor response both *in vitro* and *in vivo*. Melero, I. *et al.*, *Nature Medicine* (1997) 3:682; Kwon, E. D. *et al.*, *Proc. Natl. Acad. Sci. USA* (1997) 94: 8099; Lynch, D. H. *et al*, *Nature Medicine* (1997) 3:625; Finn, O. J. and Lotze, M. T., *J. Immunol.* (1998) 21:114. The stimulatory compounds of the invention can be administered as adjuvants, alone or together with a growth regulating agent, cytotoxic agent or chemotherapeutic agent, to stimulate T cell proliferation/activation and an antitumor response to tumor antigens. The growth regulating, cytotoxic, or chemotherapeutic agent may be administered in conventional amounts using known administration regimes. Immunostimulating activity by the compounds of the invention allows reduced amounts of the growth regulating, cytotoxic, or chemotherapeutic agents thereby potentially lowering the toxicity to the patient.

J. Screening Assays for Drug Candidates

Screening assays for drug candidates are designed to identify compounds that bind to or complex with the polypeptides encoded by the genes identified herein or a biologically active fragment thereof, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

All assays are common in that they call for contacting the drug candidate with a polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, *e.g.*, on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, *e.g.*, a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.*, the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.*, by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labelled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular protein encoded by a gene identified herein, its interaction with that protein can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature (London)* 340, 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* 88, 9578-9582 (1991)] as disclosed by Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* 89, 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for β-galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

In order to find compounds that interfere with the interaction of a gene identified herein and other intra- or extracellular components can be tested, a reaction mixture is usually prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a test compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described above. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test

compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

K. Compositions and Methods for the Treatment of Immune Related Diseases

The compositions useful in the treatment of immune related diseases include, without limitation, proteins, antibodies, small organic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple helix molecules, *etc.* that inhibit or stimulate immune function, for example, T cell proliferation/activation, lymphokine release, or immune cell infiltration.

For example, antisense RNA and RNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation initiation site, *e.g.*, between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, *e.g.*, Rossi, *Current Biology* 4, 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, *e.g.*, PCT publication No. WO 97/33551, *supra*.

These molecules can be identified by any or any combination of the screening assays discussed above and/or by any other screening techniques well known for those skilled in the art.

L. Antibodies

The present invention further provides anti-PRO antibodies and fragments thereof which may inhibit (antagonists) or stimulate (agonists) T cell proliferation, eosinophil infiltration, vascular permeability, *etc.* Such anti-PRO antibodies or fragments thereof include polyclonal, monoclonal, humanized, bispecific and heteroconjugate antibodies.

1. Polyclonal Antibodies

The anti-PRO antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

2. Monoclonal Antibodies

The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.*, 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxyapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily

isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison *et al.*, *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies are preferably monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an

"import" variable domain. Humanization can be essentially performed following the method of Winter and coworkers [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991); U.S. 5,750, 373]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *Bio/Technology* 10, 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368, 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995).

The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably 10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, humanized or human) from which the matured antibody is prepared.

4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities may be for the PRO, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 [1983]). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin

heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.*, $F(ab')_2$ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared can be prepared using chemical linkage. Brennan *et al.*, *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various technique for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148 (5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow

pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, *J. Immunol.* 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147:60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given PRO polypepide herein. Alternatively, an anti-PRO arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.*, CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular PRO polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular PRO polypeptide. These antibodies possess a PRO polypeptide -binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the PRO polypeptide and further binds tissue factor (TF).

5. Heteroconjugate Antibodies

Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

6. Effector function engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating an immune related disease, for example. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, *Anti-Cancer Drug Design* 3:219-230 (1989).

7. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain,

nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(*p*-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.* , *Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tissue pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) which is conjugated to a cytotoxic agent (*e.g.*, a radionucleotide).

8. Immunoliposomes

The proteins, antibodies, *etc.* disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl Acad. Sci. USA*, 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, *J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as doxorubicin) may be optionally contained within the liposome. See Gabizon *et al.*, *J. National Cancer Inst.* 81 (19) 1484 (1989).

M. Pharmaceutical Compositions

The active PRO molecules of the invention (*e.g.*, PRO polypeptides, anti-PRO antibodies, and/or variants of each) as well as other molecules identified by the screening assays disclosed above, can be administered for the treatment of immune related diseases, in the form of pharmaceutical compositions.

Therapeutic formulations of the active PRO molecule, preferably a polypeptide or antibody of the invention, are prepared for storage by mixing the active molecule having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences*

16th edition. Osol. A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients. or stabilizers are nontoxic to recipients at the dosages and concentrations employed. and include buffers such as phosphate. citrate, and other organic acids: antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol. butyl or benzyl alcohol: alkyl parabens such as methyl or propyl paraben: catechol; resorcinol; cyclohexanol: 3-pentanol: and m-cresol); low molecular weight (less than about 10 residues) polypeptides: proteins. such as serum albumin. gelatin. or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine. glutamine. asparagine, histidine. arginine. or lysine; monosaccharides. disaccharides, and other carbohydrates including glucose. mannose. or dextrins; chelating agents such as EDTA; sugars such as sucrose. mannitol. trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes): and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Compounds identified by the screening assays disclosed herein can be formulated in an analogous manner. using standard techniques well known in the art.

Lipofections or liposomes can also be used to deliver the PRO molecule into cells. Where antibody fragments are used. the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example. based upon the variable region sequences of an antibody. peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology (see, *e.g.*, Marasco *et al.*, *Proc. Natl. Acad. Sci. USA* 90, 7889-7893 [1993]).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated. preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition. the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active PRO molecules may also be entrapped in microcapsules prepared. for example. by coacervation techniques or by interfacial polymerization. for example. hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules. respectively, in colloidal drug delivery systems (for example. liposomes, albumin microspheres. microemulsions. nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition. Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations or the PRO molecules may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide

acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

N. Methods of Treatment

It is contemplated that the polypeptides, antibodies and other active compounds of the present invention may be used to treat various immune related diseases and conditions, such as T cell mediated diseases, including those characterized by infiltration of inflammatory cells into a tissue, stimulation of T-cell proliferation, inhibition of T-cell proliferation, increased or decreased vascular permeability or the inhibition thereof.

Exemplary conditions or disorders to be treated with the polypeptides, antibodies and other compounds of the invention, include, but are not limited to systemic lupus erythematosis, rheumatoid arthritis, juvenile chronic arthritis, osteoarthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease.

In systemic lupus erythematosus, the central mediator of disease is the production of auto-reactive antibodies to self proteins/tissues and the subsequent generation of immune-mediated inflammation. antibodies either directly or indirectly mediate tissue injury. Though T lymphocytes have not been shown to be directly involved in tissue damage, T lymphocytes are required for the development of auto-reactive antibodies. The genesis of the disease is thus T lymphocyte dependent. Multiple organs and systems are affected clinically including kidney, lung, musculoskeletal system, mucocutaneous, eye, central nervous system, cardiovascular system, gastrointestinal tract, bone marrow and blood.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte dependent and is associated with the production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of lymphocytes and monocytes into the synovium and subsequent marked synovial changes; the joint space/fluid if infiltrated by similar cells with the addition of numerous neutrophils. Tissues affected are primarily the joints, often in symmetrical pattern. However, extra-articular disease also occurs in two major forms. One form is the development of extra-articular lesions with ongoing progressive joint disease and typical lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The second form of extra-articular disease is the so called Felty's syndrome which occurs late in the RA disease course, sometimes after joint disease has become quiescent, and involves the presence of neutropenia, thrombocytopenia and splenomegaly. This can be accompanied by vasculitis in multiple organs with formations of infarcts, skin ulcers and gangrene. Patients often also develop rheumatoid nodules in the subcutis tissue overlying affected joints; the nodules late stage have necrotic centers surrounded by a mixed inflammatory cell infiltrate. Other manifestations which can occur in RA include: pericarditis, pleuritis, coronary arteritis, intestitial pneumonitis with pulmonary fibrosis, keratoconjunctivitis sicca, and rhematoid nodules.

Juvenile chronic arthritis is a chronic idiopathic inflammatory disease which begins often at less than 16 years of age. Its phenotype has some similarities to RA; some patients which are rhematoid factor positive are classified as juvenile rheumatoid arthritis. The disease is sub-classified into three major categories: pauciarticular, polyarticular, and systemic. The arthritis can be severe and is typically destructive and leads to joint ankylosis and retarded growth. Other manifestations can include chronic anterior uveitis and systemic amyloidosis.

Spondyloarthropathies are a group of disorders with some common clinical features and the common association with the expression of HLA-B27 gene product. The disorders include: ankylosing sponylitis, Reiter's syndrome (reactive arthritis), arthritis associated with inflammatory bowel disease, spondylitis associated with psoriasis, juvenile onset spondyloarthropathy and undifferentiated spondyloarthropathy. Distinguishing features include sacroileitis with or without spondylitis; inflammatory asymmetric arthritis; association with HLA-B27 (a serologically defined allele of the HLA-B locus of class I MHC); ocular inflammation, and absence of autoantibodies associated with other rheumatoid disease. The cell most implicated as key to induction of the disease is the CD8+ T lymphocyte, a cell which targets antigen presented by class I MHC molecules. CD8+ T cells may react against the class I MHC allele HLA-B27 as if it were a foreign peptide expressed by MHC class I molecules. It has been hypothesized that an epitope of HLA-B27 may mimic a bacterial or other microbial antigenic epitope and thus induce a CD8+ T cells response.

Systemic sclerosis (scleroderma) has an unknown etiology. A hallmark of the disease is induration of the skin; likely this is induced by an active inflammatory process. Scleroderma can be localized or systemic; vascular lesions are common and endothelial cell injury in the microvasculature is an early and important event in the development of systemic sclerosis; the vascular injury may be immune mediated. An immunologic basis is implied by the presence of mononuclear cell infiltrates in the cutaneous lesions and the presence of anti-nuclear antibodies in many patients. ICAM-1 is often upregulated on the cell surface of fibroblasts in skin

lesions suggesting that T cell interaction with these cells may have a role in the pathogenesis of the disease. Other organs involved include: the gastrointestinal tract: smooth muscle atrophy and fibrosis resulting in abnormal peristalsis/motility; kidney: concentric subendothelial intimal proliferation affecting small arcuate and interlobular arteries with resultant reduced renal cortical blood flow, results in proteinuria, azotemia and hypertension; skeletal muscle: atrophy, interstitial fibrosis; inflammation; lung: interstitial pneumonitis and interstitial fibrosis; and heart: contraction band necrosis, scarring/fibrosis.

Idiopathic inflammatory myopathies including dermatomyositis, polymyositis and others are disorders of chronic muscle inflammation of unknown etiology resulting in muscle weakness. Muscle injury/inflammation is often symmetric and progressive. Autoantibodies are associated with most forms. These myositis-specific autoantibodies are directed against and inhibit the function of components, proteins and RNA's, involved in protein synthesis.

Sjögren's syndrome is due to immune-mediated inflammation and subsequent functional destruction of the tear glands and salivary glands. The disease can be associated with or accompanied by inflammatory connective tissue diseases. The disease is associated with autoantibody production against Ro and La antigens, both of which are small RNA-protein complexes. Lesions result in keratoconjunctivitis sicca, xerostomia, with other manifestations or associations including bilary cirrhosis, peripheral or sensory neuropathy, and palpable purpura.

Systemic vasculitis are diseases in which the primary lesion is inflammation and subsequent damage to blood vessels which results in ischemia/necrosis/degeneration to tissues supplied by the affected vessels and eventual end-organ dysfunction in some cases. Vasculitides can also occur as a secondary lesion or sequelae to other immune-inflammatory mediated diseases such as rheumatoid arthritis, systemic sclerosis, *etc.*, particularly in diseases also associated with the formation of immune complexes. Diseases in the primary systemic vasculitis group include: systemic necrotizing vasculitis: polyarteritis nodosa, allergic angiitis and granulomatosis, polyangiitis; Wegener's granulomatosis; lymphomatoid granulomatosis; and giant cell arteritis. Miscellaneous vasculitides include: mucocutaneous lymph node syndrome (MLNS or Kawasaki's disease), isolated CNS vasculitis, Behet's disease, thromboangiitis obliterans (Buerger's disease) and cutaneous necrotizing venulitis. The pathogenic mechanism of most of the types of vasculitis listed is believed to be primarily due to the deposition of immunoglobulin complexes in the vessel wall and subsequent induction of an inflammatory response either via ADCC, complement activation, or both.

Sarcoidosis is a condition of unknown etiology which is characterized by the presence of epithelioid granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis involves the persistence of activated macrophages and lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, and paroxysmal noctural hemoglobinuria is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

In autoimmune thrombocytopenia including thrombocytopenic purpura, and immune-mediated thrombocytopenia in other clinical settings, platelet destruction/removal occurs as a result of either antibody or complement attaching to platelets and subsequent removal by complement lysis, ADCC or FC-receptor mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase).

Type I diabetes mellitus or insulin-dependent diabetes is the autoimmune destruction of pancreatic islet β cells; this destruction is mediated by auto-antibodies and auto-reactive T cells. Antibodies to insulin or the insulin receptor can also produce the phenotype of insulin-non-responsiveness.

Immune mediated renal diseases, including glomerulonephritis and tubulointerstitial nephritis, are the result of antibody or T lymphocyte mediated injury to renal tissue either directly as a result of the production of autoreactive antibodies or T cells against renal antigens or indirectly as a result of the deposition of antibodies and/or immune complexes in the kidney that are reactive against other, non-renal antigens. Thus other immune-mediated diseases that result in the formation of immune-complexes can also induce immune mediated renal disease as an indirect sequelae. Both direct and indirect immune mechanisms result in inflammatory response that produces/induces lesion development in renal tissues with resultant organ function impairment and in some cases progression to renal failure. Both humoral and cellular immune mechanisms can be involved in the pathogenesis of lesions.

Demyelinating diseases of the central and peripheral nervous systems, including Multiple Sclerosis; idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome; and Chronic Inflammatory Demyelinating Polyneuropathy, are believed to have an autoimmune basis and result in nerve demyelination as a result of damage caused to oligodendrocytes or to myelin directly. In MS there is evidence to suggest that disease induction and progression is dependent on T lymphocytes. Multiple Sclerosis is a demyelinating disease that is T lymphocyte-dependent and has either a relapsing-remitting course or a chronic progressive course. The etiology is unknown; however, viral infections, genetic predisposition, environment, and autoimmunity all contribute. Lesions contain infiltrates of predominantly T lymphocyte mediated, microglial cells and infiltrating macrophages; CD4+T lymphocytes are the predominant cell type at lesions. The mechanism of oligodendrocyte cell death and subsequent demyelination is not known but is likely T lymphocyte driven.

Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias; Idiopathic Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a disregulated immune-inflammatory response. Inhibition of that response would be of therapeutic benefit.

Autoimmune or Immune-mediated Skin Disease including Bullous Skin Diseases, Erythema Multiforme, and Contact Dermatitis are mediated by auto-antibodies, the genesis of which is T lymphocyte-dependent.

Psoriasis is a T lymphocyte-mediated inflammatory disease. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

Allergic diseases, including asthma: allergic rhinitis; atopic dermatitis: food hypersensitivity; and urticaria are T lymphocyte dependent. These diseases are predominantly mediated by T lymphocyte induced inflammation. IgE mediated-inflammation or a combination of both.

Transplantation associated diseases, including Graft rejection and Graft-Versus-Host-Disease (GVHD) are T lymphocyte-dependent; inhibition of T lymphocyte function is ameliorative.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are infectious disease including but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E and herpes) bacterial infection, fungal infections, and protozoal and parasitic infections (molecules (or derivatives/agonists) which stimulate the MLR can be utilized therapeutically, to enhance the immune response to infectious agents), diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (*i.e.*, as from chemotherapy) immunodeficiency), and neoplasia.

It has been demonstrated that some human cancer patients develop an antibody and/or T lymphocyte response to antigens on neoplastic cells. It has also been shown in animal models of neoplasia that enhancement of the immune response can result in rejection or regression of that particular neoplasm. Molecules that enhance the T lymphocyte response in the MLR have utility *in vivo* in enhancing the immune response against neoplasia. Molecules which enhance the T lymphocyte proliferative response in the MLR (or small molecule agonists or antibodies that affected the same receptor in an agonistic fashion) can be used therapeutically to treat cancer. Molecules that inhibit the lymphocyte response in the MLR also function *in vivo* during neoplasia to suppress the immune response to a neoplasm; such molecules can either be expressed by the neoplastic cells themselves or their expression can be induced by the neoplasm in other cells. Antagonism of such inhibitory molecules (either with antibody, small molecule antagonists or other means) enhances immune-mediated tumor rejection.

Additionally, inhibition of molecules with proinflammatory properties may have therapeutic benefit in reperfusion injury; stroke; myocardial infarction; atherosclerosis; acute lung injury; hemorrhagic shock; burn; sepsis/septic shock; acute tubular necrosis; endometriosis; degenerative joint disease and pancreatis.

The compounds of the present invention, *e.g.*, polypeptides or antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation (intranasal, intrapulmonary) routes. Intravenous or inhaled administration of polypeptides and antibodies is preferred.

In immunoadjuvant therapy, other therapeutic regimens, such administration of an anti-cancer agent, may be combined with the administration of the proteins, antibodies or compounds of the instant invention. For example, the patient to be treated with a the immunoadjuvant of the invention may also receive an anti-cancer agent (chemotherapeutic agent) or radiation therapy. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the immunoadjuvant or may be given simultaneously therewith. Additionally, an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see,

EP 616812) may be given in dosages known for such molecules.

It may be desirable to also administer antibodies against other immune disease associated or tumor associated antigens, such as antibodies which bind to CD20, CD11a, CD18, ErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be coadministered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. In one embodiment, the PRO polypeptides are coadministered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by a PRO polypeptide. However, simultaneous administration or administration first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and the PRO polypeptide.

For the treatment or reduction in the severity of immune related disease, the appropriate dosage of an a compound of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The compound is suitably administered to the patient at one time or over a series of treatments.

For example, depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (*e.g.*, 0.1-20 mg/kg) of polypeptide or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

O. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials (*e.g.*, comprising a PRO molecule) useful for the diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and an instruction. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is usually a polypeptide or an antibody of the invention. An instruction or label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

P. Diagnosis and Prognosis of Immune Related Disease

Cell surface proteins, such as proteins which are overexpressed in certain immune related diseases, are excellent targets for drug candidates or disease treatment. The same proteins along with secreted proteins encoded by the genes amplified in immune related disease states find additional use in the diagnosis and

prognosis of these diseases. For example, antibodies directed against the protein products of genes amplified in multiple sclerosis, rheumatoid arthritis, or another immune related disease, can be used as diagnostics or prognostics.

For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of proteins encoded by amplified or overexpressed genes ("marker gene products"). The antibody preferably is equipped with a detectable, *e.g.*, fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These techniques are particularly suitable, if the overexpressed gene encodes a cell surface protein Such binding assays are performed essentially as described above.

In situ detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA. Unless otherwise noted, the present invention uses standard procedures of recombinant DNA technology, such as those described hereinabove and in the following textbooks: Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press N.Y., 1989; Ausubel *et al.*, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., 1989; Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press, inc., N.Y., 1990; Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, 1988; Gait, M.J., *Oligonucleotide Synthesis*, IRL Press, Oxford, 1984; R.I. Freshney, *Animal Cell Culture*, 1987; Coligan *et al.*, *Current Protocols in Immunology*, 1991.

EXAMPLE 1

Isolation of cDNA clones Encoding Human PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325,

PRO1338. PRO1434. PRO4333. PRO4302. PRO4430 and PRO5727 polypeptide.

Various techniques were employed for isolating the cDNA clones described below. A general description of the methods employed follows immediately hereafter. whereas the details relating the specific sequences isolated is recited separately for each native sequence. It is understood that the actual sequences of the PRO polypeptides are those which are contained within or encoded by the clone deposited with the ATCC - and that in the in event of any discrepancy between the sequence deposited and the sequence disclosed herein. the sequence of the deposit is the true sequence

ECD Homology:

The extracellular domain (ECD) sequences (including the secretion signal sequence. if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public EST databases (*e.g.*, GenBank). a private EST database (LIFESEQ™, Incyte Pharmaceuticals. Palo Alto. CA), and proprietary ESTs from Genentech. The search was performed using the computer program BLAST or BLAST2 [Altschul *et al.*, *Methods in Enzymology*. 266: 460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons resulting in a BLAST score of 70 (or in some cases. 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green. University of Washington. Seattle. Washington).

Using various ESTs. drawing from both public and private databases. a consensus DNA sequence was assembled. Oligonucleotides were then synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone encoding the particular native sequence PRO polypeptide identified herein.

In order to screen several libraries for a source of a full-length, native sequence clone. DNA from the libraries was screened by PCR amplification with the PCR primer pair identified below. A positive library was then used to isolate clones encoding the particular native sequence PRO polypeptide using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from various human tissue libraries. including, *e.g.*, fetal lung, fetal liver. fetal brain. small intestine. smooth muscle cells. *etc.* The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors. cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see. Holmes *et al.*, *Science*. 253:1278-1280 (1991)) in the unique XhoI and NotI sites. The clones were sequenced using known and readily available methodology.

Amylase yeast screen:

1. Preparation of oligo dT primed cDNA library

mRNA was isolated from various tissues (*e.g.*, such as those indicated above under the ECD homology procedure) using reagents and protocols from Invitrogen, San Diego, CA (Fast Track 2). This RNA was used to

generate an oligo dT primed cDNA library in the vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). In this procedure, the double stranded cDNA was sized to greater than 1000 bp and the SalI/NotI linkered cDNA was cloned into XhoI/NotI cleaved vector. pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites.

2. Preparation of random primed cDNA library

A secondary cDNA library was generated in order to preferentially represent the 5' ends of the primary cDNA clones. Sp6 RNA was generated from the primary library (described above), and this RNA was used to generate a random primed cDNA library in the vector pSST-AMY.0 using reagents and protocols from Life Technologies (Super Script Plasmid System, referenced above). In this procedure the double stranded cDNA was sized to 500-1000 bp, linkered with blunt to NotI adaptors, cleaved with SfiI, and cloned into SfiI/NotI cleaved vector. pSST-AMY.0 is a cloning vector that has a yeast alcohol dehydrogenase promoter preceding the cDNA cloning sites and the mouse amylase sequence (the mature sequence without the secretion signal) followed by the yeast alcohol dehydrogenase terminator, after the cloning sites. Thus, cDNAs cloned into this vector that are fused in frame with amylase sequence will lead to the secretion of amylase from appropriately transfected yeast colonies.

3. Transformation and Detection

DNA from the library described in paragraph 2 above was chilled on ice to which was added electrocompetent DH10B bacteria (Life Technologies, 20 ml). The bacteria and vector mixture was then electroporated as recommended by the manufacturer. Subsequently, SOC media (Life Technologies, 1 ml) was added and the mixture was incubated at 37°C for 30 minutes. The transformants were then plated onto 20 standard 150 mm LB plates containing ampicillin and incubated for 16 hours (37°C). Positive colonies were scraped off the plates and the DNA was isolated from the bacterial pellet using standard protocols, *e.g.*, Cisco-gradient. The purified DNA was then carried on to the yeast protocols below.

The yeast methods were divided into three categories: (1) Transformation of yeast with the plasmid/cDNA combined vector; (2) Detection and isolation of yeast clones secreting amylase; and (3) PCR amplification of the insert directly from the yeast colony and purification of the DNA for sequencing and further analysis.

The yeast strain used was HD56-5A (ATCC-90785). This strain has the following genotype: MAT alpha, ura3-52, leu2-3, leu2-112, his3-11, his3-15, MAL^+, SUC^-, GAL^+. Preferably, yeast mutants can be employed that have deficient post-translational pathways. Such mutants may have translocation deficient alleles in *sec*71, *sec*72, *sec*62, with truncated *sec*71 being most preferred. Alternatively, antagonists (including antisense nucleotides and/or ligands) which interfere with the normal operation of these genes, other proteins implicated in this post translation pathway (*e.g.*, SEC61p, SEC72p, SEC62p, SEC63p, TDJ1p or SSA1p-4p) or the complex formation of these proteins may also be preferably employed in combination with the amylase-expressing yeast.

Transformation was performed based on the protocol outlined by Gietz *et al., Nucl. Acid. Res.*, 20:1425 (1992). Transformed cells were then inoculated from agar into YEPD complex media broth (100 ml) and

grown overnight at 30°C. The YEPD broth was prepared as described in Kaiser *et al., Methods in Yeast Genetics*, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 207 (1994). The overnight culture was then diluted to about 2 x 10^6 cells/ml (approx. OD_{600} = 0.1) into fresh YEPD broth (500 ml) and regrown to 1 x 10^7 cells/ml (approx. OD_{600}=0.4-0.5).

The cells were then harvested and prepared for transformation by transfer into GS3 rotor bottles in a Sorval GS3 rotor at 5,000 rpm for 5 minutes, the supernatant discarded, and then resuspended into sterile water, and centrifuged again in 50 ml falcon tubes at 3,500 rpm in a Beckman GS-6KR centrifuge. The supernatant was discarded and the cells were subsequently washed with LiAc/TE (10 ml, 10 mM Tris-HCl, 1 mM EDTA pH 7.5, 100 mM Li20OCCH3), and resuspended into LiAc/TE (2.5 ml).

Transformation took place by mixing the prepared cells (100 µl) with freshly denatured single stranded salmon testes DNA (Lofstrand Labs, Gaithersburg, MD) and transforming DNA (1 µg, vol. < 10 µl) in microfuge tubes. The mixture was mixed briefly by vortexing, then 40% PEG/TE (600 µl, 40% polyethylene glycol-4000, 10 mM Tris-HCl, 1 mM EDTA, 100 mM Li2Ac, pH 7.5) was added. This mixture was gently mixed and incubated at 30°C while agitating for 30 minutes. The cells were then heat shocked at 42°C for 15 minutes, and the reaction vessel centrifuged in a microfuge at 12,000 rpm for 5-10 seconds, decanted and resuspended into TE (500 µl, 10 mM Tris-HCl, 1 mM EDTA pH 7.5) followed by recentrifugation. The cells were then diluted into TE (1 ml) and aliquots (200 µl) were spread onto the selective media previously prepared in 150 mm growth plates (VWR).

Alternatively, instead of multiple small reactions, the transformation was performed using a single, large scale reaction, wherein reagent amounts were scaled up accordingly.

The selective media used was a synthetic complete dextrose agar lacking uracil (SCD-Ura) prepared as described in Kaiser *et al., Methods in Yeast Genetics*, Cold Spring Harbor Press, Cold Spring Harbor, NY, p. 208-210 (1994). Transformants were grown at 30°C for 2-3 days.

The detection of colonies secreting amylase was performed by including red starch in the selective growth media. Starch was coupled to the red dye (Reactive Red-120, Sigma) as per the procedure described by Biely *et al., Anal. Biochem.*, 172:176-179 (1988). The coupled starch was incorporated into the SCD-Ura agar plates at a final concentration of 0.15% (w/v), and was buffered with potassium phosphate to a pH of 7.0 (50-100 mM final concentration).

The positive colonies were picked and streaked across fresh selective media (onto 150 mm plates) in order to obtain well isolated and identifiable single colonies. Well isolated single colonies positive for amylase secretion were detected by direct incorporation of red starch into buffered SCD-Ura agar. Positive colonies were determined by their ability to break down starch resulting in a clear halo around the positive colony visualized directly.

Isolation and sequencing by standard techniques identified a yeast EST fragment which served as the basis for additional database mining as described below.

4. Assembly

The yeast EST fragment identified above was used to search various expressed sequence tag (EST) databases. The EST databases included public EST databases (*e.g.*, GenBank, Merck/Wash U) and a proprietary EST DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA). The search was

performed using the computer program BLAST or BLAST2 (Altshul *et al., Methods in Enzymology* 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. The consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above as well as EST sequences proprietary to Genentech.

Based on this consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone encoding the particular PRO polypeptide. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al., Current Protocols in Molecular Biology,* with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

RNA for construction of the cDNA libraries was isolated from various human tissues. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; Holmes *et al., Science,* 253: 1278-1280 (1991)) in the unique XhoI and NotI sites.

Signal algorithm:

A proprietary signal sequence finding algorithm developed by Genentech, Inc was used upon Expressed Sequence Tags (ESTs) and on clustered and assembled EST fragments from public (*e.g.*, GenBank) and/or private (Lifeseq®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals.

The above procedure resulted in the identification of EST sequences which were compared to a variety of expressed sequence tag (EST) databases which included public EST databases (*e.g.*, GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The homology search was performed using the computer program BLAST or BLAST2 (Altshul *et al., Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater

that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). This resulted in the identification of additional EST sequences which either corresponded to full-length clones, which were examined and sequenced or served as a template for the creation of cloning oligonucleotides which were then used to screen various tissue libraries resulting in isolation of DNA encoding a native sequence PRO polypeptide.

A. Isolation of cDNA clones Encoding Human PRO200 (UNQ174)

Probes based on an expressed sequence tag (EST) identified from the Incyte Pharmaceuticals database due to homology with VEGF were used to screen a cDNA library derived from the human glioma cell line G61. Screening may be conducted in a manner similar to the procedure disclosed elsewhere in this application. In particular, Incyte Clone "INC1302516" was used to generate the following four probes:

5'-ACTTCTCAGTGTCCATAAGGG-3' (SEQ ID NO:3)
5'-GAACTAAAGAGAACCGATACCATTTTCTGGCCAGGTTGTC-3' (SEQ ID NO:4)
5'-CACCACAGCGTTTAACCAGG-3' (SEQ ID NO:5)
5'-ACAACAGGCACAGTTCCCAC-3' (SEQ ID NO:6)

Nine positives were identified and characterized. Three clones contained the full coding region and were identical in sequence. Partial clones were also identified from a fetal lung library and were identical with the glioma-derived sequence with the exception of one nucleotide change which did not alter the encoded amino acid.

For mammalian protein expression, the entire open reading frame (ORF) was cloned into a CMV-based expression vector. An epitope-tag (FLAG, Kodak) and Histidine-tag (His8) were inserted between the ORF and stop codon. UNQ174-His8 and UNQ174-FLAG were transfected into human embryonic kidney 293 cells by SuperFect (Qiagen) and pulse-labeled for 3 hours with [^{35}S]methionine and [^{35}C]cysteine. Both epitope-tagged proteins co-migrate when 20 microliters of 15-fold concentrated serum-free conditioned medium were electrophoresed on a polyacrylamide gel (Novex) in sodium dodecyl sulfate sample buffer (SDS-PAGE). The UNQ174-IgG expression plasmid was constructed by cloning the ORF in front of the human Fc (IgG) sequence.

The UNQ174-IgG plasmid was co-transfected with Baculogold Baculovirus DNA (Pharmingen) using Lipofectin (GibcoBRL) into 10^5 Sf9 cells grown in Hink's TNM-FH medium (JRH Biosciences) supplemented with 10% fetal bovine serum. Cells were incubated for 5 days at 28°C. The supernatant was harvested and subsequently used for the first viral amplification by infecting Sf9 cells at an approximate multiplicity of infection (MOI) of 10. Cells were incubated for 3 days, then supernatant harvested, and expression of the recombinant plasmid determined by binding of 1 ml of supernatant to 30 µl of Protein-A Sepharose CL-4B beads (Pharmacia) followed by subsequent SDS-PAGE analysis. The first amplification supernatant was used to infect a 500 ml spinner culture of Sf9 cells grown in ESF-921 medium (Expression Systems LLC) at an approximate MOI of 0.1. Cells were treated as above, except harvested supernatant was sterile filtered. Specific protein was purified by binding to Protein-A Sepharose 4 Fast Flow (Pharmacia) column.

The entire nucleotide sequence of the identified clone DNA29101 is shown in Figure 1 (SEQ ID NO:1). Clone DNA29101 (SEQ ID NO:1) contains a single open reading frame with an apparent translation initiation site at nucleotide residues 285-287 and ending at the stop codon (TAG) found at nucleotide positions 1320-1322 (Figure 1, SEQ ID NO:1), as indicated by bolded underline. The predicted PRO200 polypeptide precursor (*i.e.*, UNQ174, SEQ ID NO:2) is 345 amino acids in length, has a calculated molecular weight of 39029 daltons, a pI of 6.06 and is shown in Figure 2 (SEQ ID NO:2). Potential N-glycosylation sites are at amino acid residues 25, 54 and 254. CUB domains are at amino acid residues 52-65, 118-125 and 260-273.

A cDNA containing DNA encoding UNQ174 (SEQ ID NO:2) has been deposited with the ATCC on March 5, 1998 and has been assigned deposit number 209653.

B. Isolation of cDNA clones Encoding Human PRO204 (UNQ178)

An expressed sequence tag (EST) DNA database (LIFESEQ℠, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST was identified. Human fetal retina cDNA libraries were screened with PCR

oligonucleotide primers and confirmed by hybridization with synthetic oligonucleotide probe which was based upon the EST sequence.

hybridization probe:

5'-GGCATGCAGCAGCTGGACATTTGCGAGGGCTTTTGCTGGCTG-3' (SEQ ID NO:7)

forward PCR primer:

5'-CTGCTGCAGAGTTGCACGAAC-3' (SEQ ID NO:8)

reverse PCR primer 1:

5'-CAGTTGTTGTTGTCACAGAGAAG-3' (SEQ ID NO:9)

reverse PCR primer 2:

5'-AGTTCGTGCAACTCTGCAGCAG-3' (SEQ ID NO:10)

A cDNA clone was identified and sequenced in entirety. The entire nucleotide sequence of the identified clone DNA30871 is shown in Figure 3 (SEQ ID NO:11). Clone DNA30871-1157 (SEQ ID NO:11) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 376-378 and ending at the stop codon (TAA) found at nucleotide positions 1498-1500 (Figure 3; SEQ ID NO:11), as indicated by bolded underline. The predicted PRO204 polypeptide precursor (i.e., UNQ178, SEQ ID NO:12) is 374 amino acids long, has a calculated molecular weight of 39,285 daltons, a pI of 6.06 and is shown in Figure 4. A cDNA containing DNA encoding UNQ178 (SEQ ID NO:12) has been deposited with the ATTC on October 16, 1997 and has been assigned deposit number 209380.

C. Isolation of cDNA clones Encoding Human PRO212 (UNQ186)

Use of the ECD homology procedure described above from a human fetal lung library resulted in the identification of the full-length DNA sequence for DNA30942 (Fig. 5; SEQ ID NO:13) and the derived protein sequence UNQ186 (Fig. 6; SEQ ID NO:14).

The PCR primers (forward and reverse) and probes used in the procedure were the following:

forward primer: 5'-CACGCTGGTTTCTGCTTGGAG-3' (SEQ ID NO:15)

reverse primer: 5'-AGCTGGTGCACAGGGTGTCATG-3' (SEQ ID NO:16)

hybridization probe: (SEQ ID NO:17)

5'-CCCAGGCACCTTCTCAGCCAGCCAGCAGCTCCAGCTCAGAGCAGTGCCAGCCC-3'

The entire nucleotide sequence of DNA30942 is shown in Figure 5 (SEQ ID NO:13). Clone DNA30942 (SEQ ID NO:13) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 101-103 and ending at the stop codon (TGA) at positions 1001-1003 (Fig. 5; SEQ ID NO:13), as indicated in bolded underline. The predicted PRO212 polypeptide precursor of Fig. 6 (SEQ ID NO:14) is 300 amino acids long, has a calculated molecular weight of 32680 daltons and a pI of 8.70. It is believed that the PRO212 sequence of Fig. 6 (SEQ ID NO:14) lacks a transmembrane domain. It is also believed that amino acids 1 to 215 of Fig. 6 (SEQ ID NO:14) represents an ECD which includes four cysteine rich domains (CRDs). A cDNA clone containing DNA30942 (SEQ ID NO:13) has been deposited with ATCC (identified as DNA30942-1134) on September 16, 1997 and has been assigned ATCC deposit no. 209254.

D. Isolation of cDNA clones Encoding Human PRO216 (UNQ190)

A procedure analogous to the one above for the isolation of PRO212 can be employed to isolate DNA33087 (SEQ ID NO:18) (Figure 7) which encodes the PRO216 polypeptide UNQ190 (SEQ ID NO:19)(Figure 8).

DNA33087 contains a single open reading frame with an apparent translation initiation site nucleotide residues 268-270 and ending at the stop codon (TAG) are residues 1531-1533 (Fig. 7, SEQ ID NO:18), as indicated by bolded underline. The predicted PRO215 polypeptide precursor (*i.e.*, UNQ190, SEQ ID NO:19) is 421 amino acids long, has a calculated molecular weight of 49492 daltons and a pI of 5.51 (Fig. 8).

Hydropathy analysis suggests the presence of a signal sequence at amino acid residues 1 to 20, tyrosine kinase phosphorylation sites at amino acid residues 268-274 and 300-306, and N-myristoylation site residue 230-235, and leucine zippers at residues 146 to 167 and 217 to 238. Alternatively to traditional isolation techniques, the DNA sequence is publicly available from GenBank as accession number AB000114 which encodes Dayhoff protein AB000114_1.

Alternatively still, the sequence is described in *Ohno et al., Biochem. Biophys. Res. Commun.* 228(2): 411-414 (1996). A cDNA clone containing DNA33087 (identified as DNA33087-1158) has been deposited with the American Type Culture Collection (ATCC) on September 16, 1997 and has been assigned ATCC Dep. No. 209381.

E. Isolation of cDNA clones Encoding Human PRO226 (UNQ200)

Use of the ECD homology procedure described above in a human fetal lung library resulted in the identification of the full-length DNA sequence for DNA33460 (Figure 9; SEQ ID NO:20) and the derived native sequence protein UNQ200 (SEQ ID NO:21).

DNA33460 contains a single open reading frame with an apparent translation initiation site at nucleotide residues 62-64 and ending at the stop codon (TGA) at residues 1391-1393 (Fig. 9; SEQ ID NO: 20), as indicated by bolded underline. The predicted PRO226 polypeptide precursor (*i.e.*, UNQ200, SEQ ID NO:21) is 443 amino acids long, has a calculated molecular weight of 49,391 daltons, a pI of 4.82 and is shown in Figure 10 as UNQ200 (SEQ ID NO:21). A cDNA clones containing DNA33460 (SEQ ID NO:20), designated as DNA33460-1166, has been deposited with the ATCC on October 16, 1997 and has been assigned ATCC deposit number 209376.

The oligonucleotide sequences used in the above procedure were the following:

28722.p (OLI488) (SEQ ID NO: 22)
5'-TGTGTGGACATAGACGAGTGCCGCTACCGCTACTGCCAGCACCGC-3'

28722.f (OLI489) (SEQ ID NO: 23)
5'-AGGACTGCCATAACTTGCCTG-3'

28722.r (OLI490) (SEQ ID NO: 24)
5'-ATAGGAGTTGAAGCAGCGCTGC-3'

F. Isolation of cDNA clones Encoding Human PRO240 (UNQ214)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the isolation of the full-length DNA sequence for DNA34387 (Figure 11; SEQ ID NO:25) and the derived native sequence protein UNQ214 (SEQ ID NO:26).

The entire nucleotide sequence of DNA34387 is shown in Figure 11 (SEQ ID NO:25). The clone DNA34387 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 12-14 and ending at the stop codon (TGA) at nucleotide positions 699-701 (Fig. 11; SEQ ID NO:25), as indicated by bolded underline. The predicted PRO240 polypeptide precursor (*i.e.*, UNQ214, SEQ ID NO:26) is 229 amino acids long, has a calculated molecular weight of 24.689 daltons, a pI of 7.83 and is shown in Figure 12. A cDNA clone containing DNA34387 (SEQ ID NO:25) has been deposited with ATCC on September 16, 1997 and is assigned ATCC deposit no. 209260.

The PCR primers (forward and reverse) and hybridization probe synthesized for use in the above-described procedure were the following:

forward PCR primer:	5'-TCAGCTCCAGACTCTGATACTGCC-3'	(SEQ ID NO:27)
reverse PCR primer:	5'-TGCCTTTCTAGGAGGCAGAGCTCC-3'	(SEQ ID NO:28)
hybridization probe:		(SEQ ID NO:29)

5'-GGACCCAGAAATGTGTCCTGAGAATGGATCTTGTGTACCTGATGGTCCAG-3'

G. Isolation of cDNA clones Encoding Human PRO235 (UNQ209)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the isolation of the full-length DNA sequence for DNA35558 (Figure 13; SEQ ID NO:30) and the derived PRO235 native sequence protein UNQ209 (Fig. 14, SEQ ID NO:31).

The entire nucleotide sequence of DNA35558 is shown in Figure 13 (SEQ ID NO:30). The DNA35558 clone shown in Figure 13 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 667-669 and ending at the stop codon (TGA) at nucleotide positions 2323-2325, as indicated by bolded underline. The predicted PRO235 polypeptide precursor (*i.e.*, UNQ209, SEQ ID NO:31) is 552 amino acids long, has a calculated molecular weight of 61,674 daltons and a pI of 6.95 (Figure 14). A cDNA clone containing DNA35558 has been deposited with ATCC on October 16, 1997 and is assigned ATCC deposit no. 209374.

The PCR primers (forward and reverse) and hybridization probe synthesized for use in the above procedure were:

forward PCR primer:	5'-TGGAATACCGCCTCCTGCAG-3'	(SEQ ID NO:32)
reverse PCR primer:	5'-CTTCTGCCCTTTGGAGAAGATGGC-3'	(SEQ ID NO:33)
hybridization probe:		
5'-GGACTCACTGGCCCAGGCCTTCAATATCACCAGCCAGGACGAT-3'		(SEQ ID NO:34)

H. Isolation of cDNA Clones Encoding Human PRO245 (UNQ219)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the isolation of the full-length DNA sequence for DNA35658 (Figure 15, SEQ ID NO:35) and the derived PRO245 native sequence protein UNQ219 (Figure 16, SEQ ID NO:36).

The PCR primers (forward and reverse) and hybridization probes synthesized for use with the above-described method were the following:

forward PCR primer	5'-ATCGTTGTGAAGTTAGTGCCCC-3'	(SEQ ID NO:37)
reverse PCR primer	5'-ACCTGCGATATCCAACAGAATTG-3'	(SEQ ID NO:38)
hybridization probe		(SEQ ID NO:39)

5'-GGAAGAGGATACAGTCACTCTGGAAGTATTAGTGGCTCCAGCAGTTCC-3'

The entire nucleotide sequence of DNA35638 (SEQ ID NO:35) is shown in Figure 15. Clone DNA35638 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 89-91 and ending at the stop codon (TAG) at nucleotide positions 1025-1027 (Fig. 15; SEQ ID NO:35). The predicted PRO245 polypeptide precursor (*i.e.*, UNQ219, SEQ ID NO:36) is 312 amino acids long, has a calculated molecular weight of 34,554 daltons and a pI of 9.39 (Fig. 36). A clone containing DNA35638 (SEQ ID NO:35), designated as DNA35638-1141, has been deposited with ATCC on September 16, 1997 and is assigned ATCC deposit no. 209265.

I. Isolation of cDNA clones Encoding Human PRO172 (UNQ146)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the isolation of the full-length DNA sequence for DNA35916 (Fig. 17; SEQ ID NO:40) and the derived PRO172 native sequence protein UNQ146 (Fig. 18, SEQ ID NO:41).

Clone DNA35916 (SEQ ID NO:40) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 38-40 and ending at the stop codon (TAA) at nucleotide positions 2207-2209, as indicated by bolded underline in Fig. 17. The predicted PRO172 polypeptide precursor (*i.e.*, UNQ146; SEQ ID NO:41) is 723 amino acids long, has a calculated molecular weight of 78,055 daltons and a pI of 6.17 (Fig. 18). A cDNA clone containing DNA35916 (SEQ ID NO:40) has been deposited with ATCC on October 28, 1997 (designated as DNA35916-1161) and has been assigned ATCC deposit no. 209419.

The oligonucleotide sequences used in the above procedure were the following:

28765.p (OLI633)
5'-AAATCTGTGAATTGAGTGCCATGGACCTGTTGCGGACGGCCCTTGCTT-3' (SEQ ID NO:42)

28765.f (OLI644)
5'-GGATCTCGAGAACAGCTACTCC-3' (SEQ ID NO:43)

28765.r (OLI645)
5'-TCGTCCACGTTGTCGTCACATG-3' (SEQ ID NO:44)

J. Isolation of cDNA clones Encoding Human PRO273 (UNQ240)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the isolation of the full-length DNA sequence for DNA39523 (Fig. 19; SEQ ID NO:45) and the derived PRO273 native sequence protein UNQ240 (Fig. 20, SEQ ID NO:46).

The PCR primers (forward and reverse) and hybridization probe synthesized were the following:

forward PCR primer: 5'-CAGCGCCCTCCCCATGTCCCTG-3' (SEQ ID NO:47)
reverse PCR primer: 5'-TCCCAACTGGTTTGGAGTTTTCCC-3' (SEQ ID NO:48)
hybridization probe:
5'-CTCCGGTCAGCATGAGGCTCCTGGCGGCCGCTGCTCCTGCTGCTG-3' (SEQ ID NO:49)

Clone DNA39523 (SEQ ID NO:45) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 167-169 and ending at the stop codon (TAG) at nucleotide positions 500-502 (Figure 19), as indicated by bolded underline. The predicted PRO273 polypeptide precursor (*i.e.*, UNQ240, SEQ ID NO:46) is 111 amino acids long, has a calculated molecular weight of 13,078 daltons and a pI of 10.37 (Figure 20). A cDNA clone including DNA39523 (SEQ ID NO:45) has been deposited with ATCC on October 31. 1997 and is assigned ATCC deposit no. 209424.

K. Isolation of cDNA clones Encoding Human PRO272 (UNQ239)

Use of the ECD homology procedure described above in a human fetal lung tissue in combination with an *in vivo* cloning procedure using the probe oligonucleotide and one of the primer pairs resulted in the identification of the full length DNA sequence for DNA40620 (Fig. 21, SEQ ID NO:50) and the derived PRO272 native sequence protein UNQ239 (SEQ ID NO:51).

The forward and reverse PCR primers and hybridization probes synthesized and used to isolate the PRO272 encoding DNA sequences were the following:

forward PCR primer (.f1): 5'-CGCAGGCCCTCATGGCCAGG-3' (SEQ ID NO:52)
forward PCR primer (.f2): 5'-GAAATCCTGGGTAATTGG-3' (SEQ ID NO:53)
reverse PCR primer: 5'-GTGCGCGGTGCTCACAGCTCATC-3' (SEQ ID NO:54)
hybridization probe:
5'-CCCCCCTGAGCGACGCTCCCCCATGATGACGCCCACGGGAACTTC-3' (SEQ ID NO:55)

Clone DNA40620 (SEQ ID NO:50) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 35-37 and ending at the stop codon (TGA) at nucleotide positions 1020-1022 (Figure 21), as indicated by bolded underline. The predicted polypeptide precursor is 328 amino acids long (Figure 22), has a calculated molecular weight of 37,493 daltons and a pI of 4.77. A cDNA clone containing DNA40620 (SEQ ID NO:50) has been deposited with ATCC on October 17, 1997 and is assigned ATCC deposit no. 209388.

L. Isolation of cDNA clones Encoding Human PRO332 (UNQ293)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the identification of the full-length DNA sequence for DNA40982 (Fig. 23, SEQ ID NO:56) and the derived PRO332 native sequence protein UNQ293 (Fig. 24, SEQ ID NO:57).

The PCR primers (forward and reverse) and hybridization probe synthesized for use in the above procedure were:

5'-GCATTGGCCGCGAGACTTTGCC-3' (SEQ ID NO:58)

5'-GCGGCCACGGTCCTTGGAAATG-3' (SEQ ID NO:59)

5'-TGGAGGAGCTCAACCTCAGCTACAACCGCATCACCAGCCCACAGG-3' (SEQ ID NO:60)

The entire nucleotide sequence of DNA40982 (SEQ ID NO:56) is shown in Figure 23. Clone DNA40982 (SEQ ID NO:56) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 342-344 and ending at the stop codon (TAG) at nucleotide positions 2268-2270, as indicated in Figure 23 by bolded underline. The predicted PRO332 polypeptide precursor (*i.e.*, UNQ293, SEQ ID NO:57, Fig. 24) is 642 amino acids long, and has a calculated molecular weight of 72,067, and a pI of 6.60. A cDNA clone containing DNA40982 (SEQ ID NO:56) (designated as DNA40982-1235) has been deposited with ATCC on November 7, 1997 and is assigned ATCC deposit no. 209433.

M. Isolation of cDNA clones Encoding Human PRO526 (UNQ330)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the identification of the full-length DNA sequence DNA44184 (Fig. 25, SEQ ID NO:61) and the derived PRO526 native sequence protein UNQ330 (Fig. 26, SEQ ID NO:62).

The PCR primers (forward and reverse) and hybridization probes synthesized were the following:

forward PCR primer: 5'-TGGCTGCCCTGCAGTACCTCTACC-3' (SEQ ID NO:63)

reverse PCR primer: 5'-CCCTGCAGGTCATTGGCAGCTAGG-3' (SEQ ID NO:64)

hybridization probe: (SEQ ID NO:65)

5'-AGGCACTGCCTGATGACACCTTCCGCGACCTGGGCAACCTCACAC-3'.

Clone DNA44184 (SEQ ID NO:61) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 514-516 and ending at the stop codon (TGA) at nucleotide positions 1933-1935 (Figure 61), as indicated by bolded underline. The predicted PRO526 polypeptide precursor (*i.e.*, UNQ330, SEQ ID NO:62) is 473 amino acids long (Figure 62). The UNQ330 (SEQ ID NO:62) protein shown in Figure 62 has an estimated molecular weight of about 50708 daltons and a pI of about 9.28. A cDNA clone containing DNA44184 has been deposited with the ATCC on 26 March 1998 (under the designation DNA44184-1319) and is assigned deposit number 209704.

Analysis of UNQ330 (SEQ ID NO:62) revels that the signal peptide sequence is at about amino acids 1-26. A leucine zipper pattern is at about amino acids 135-156. A glycosaminoglycan attachment is at about amino acids 436-439. N-glycosylation sites are at about amino acids 82-85, 179-182, 237-240 and 423-426. A von Willebrand factor (VWF) type C domain(s) is found at about amino acids 411-425. The skilled artisan can understand which nucleotides correspond to these amino acids based on the sequences provided herein.

N. Isolation of cDNA clones Encoding Human PRO701 (UNQ365)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the identification of the full-length DNA sequence DNA44205 (Fig. 27, SEQ ID NO:66) and the derived PRO526 native sequence protein UNQ365 (Fig. 28, SEQ ID NO:67).

The PCR primers (forward and reverse) and hybridization probe synthesized for use in the above procedure were:

5'-GGCAAGCTACGGAAACGTCATCGTG-3' (SEQ ID NO:68)
5'-AACCCCCGAGCCAAAAGATGGTCAC-3' (SEQ ID NO:69)
5'-GTACCGGTGACCAGGCAGCAAAAGGCAACTATGGGCTCCTGGATCAG-3' (SEQ ID NO:70)

Clone DNA44205 (SEQ ID NO:66) contains a single open reading frame (with an apparent translation initiation site at nucleotide positions 50-52 and ending at the stop codon (TAG) at nucleotide positions 2498-3000, as indicated by bolded underline in Figure 27. The predicted PRO701 polypeptide precursor (*i.e.*, Fig. 28, UNQ365, SEQ ID NO:67) is 816 amino acids long, and has a calculated molecular weight of 91,794 Da (pI: 5.88). A cDNA clone containing DNA44205 (SEQ ID NO:66) (designated as DNA44205-1285) has been deposited with ATCC on March 31, 1998 and is assigned ATCC deposit no. 209720.

UNQ365 (SEQ ID NO:67) contains a potential signal peptide cleavage site at about amino acid position 25. There are potential N-glycosylation sites at about amino acid positions 83, 511, 716 and 803. The carboxylesterases type-B signature 2 sequence is at about residues 125 to 135. Regions homologous with carboxylesterase type-B are also at about residues 54-74, 197-212 and 221-261. A potential transmembrane region corresponds approximately to amino acids 671 through about 700. The corresponding nucleic acids can be routinely determined from the sequences provided herein.

O. Isolation of cDNA clones Encoding Human PRO361 (UNQ316)

Use of the ECD homology procedure described above in combination with an *in vivo* cloning procedure using the probe oligonucleotide and one of the primer pairs in a human fetal kidney library resulted in the identification of the full-length DNA sequence DNA45410 (Fig. 29, SEQ ID NO:71) and the derived PRO361 native sequence protein UNQ316 (Fig. 30, SEQ ID NO:72).

The forward and reverse PCR primers and a hybridization probe were synthesized for use in the above-described method:

forward PCR primer (.f1):
5'-AGGGAGGATTATCCTTGACCTTTGAAGACC-3' (SEQ ID NO:73)
forward PCR primer (.f2): 5'-GAAGCAAGTGCCCAGCTC-3' (SEQ ID NO:74)
forward PCR primer (.f3): 5'-CGGGTCCCTGCTCTTTGG-3' (SEQ ID NO:75)
reverse PCR primer (.r1): 5'-CACCGTAGCTGGGAGCGCACTCAC-3' (SEQ ID NO:76)
reverse PCR primer (.r2): 5'-AGTGTAAGTCAAGCTCCC-3' (SEQ ID NO:77)
hybridization probe:
5'- GCTTCCTGACACTAAGGCTGTCTGCTAGTCAGAATTGCCTCAAAAAGAG-3' (SEQ ID NO:78)

Clone DNA45410 (SEQ ID NO:71) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 226-228 and ending at the stop codon (TAA) at nucleotide positions 1519-1521 (Figure 29), as indicated by bolded underline. The predicted PRO361 polypeptide precursor (*i.e.*, UNQ316, SEQ ID NO:72) is 431 amino acids long (Figure 30). The native sequence PRO361 protein shown in Figure 30 as UNQ316 has an estimated molecular weight of about 46810 and a pI of about 6.45. In addition, regions indicative of the arginase family proteins are present at about residues F3 to V14 and again at I39 to T57, while a transmembrane domain exists at about residues P380 to S409. A cDNA clone containing

DNA45410 (SEQ ID NO:71) has been deposited with ATCC on February 5, 1998 and is assigned ATCC deposit no. 209621.

P. Isolation of cDNA clones Encoding Human PRO362 (UNQ317)

Use of the ECD homology procedure described above in a human fetal brain library resulted in the isolation of the full-length DNA sequence DNA45416 (Fig. 31, SEQ ID NO:79) and the derived PRO362 native sequence protein UNQ317 (Fig. 32, SEQ ID NO:80).

The PCR primers (forward and reverse) and hybridization probe synthesized for use in the above procedure were:

forward PCR primer 1:	5'-TATCCCTCCAATTGAGCACCCTGG-3'	(SEQ ID NO:81)
forward PCR primer 2:	5'-GTCGGAAGACATCCCAACAAG-3'	(SEQ ID NO:82)
reverse PCR primer 1:	5'-CTTCACAATGTCGCTGTGCTGCTC-3'	(SEQ ID NO:83)
reverse PCR primer 2:	5'-AGCCAAATCCAGCAGCTGGCTTAC-3'	(SEQ ID NO:84)

hybridization probe:

5'-TGGATGACCGGAGCCACTACACGTGTGAAGTCACCTGGCAGACTCCTGAT-3' (SEQ ID NO:85)

Clone DNA45416 (SEQ ID NO:79) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 119-121 and ending at the stop codon (TAA) at nucleotide positions 1082-1084 (Figure 31), as indicated by bolded underline. The predicted PRO362 polypeptide precursor (*i.e.*, UNQ317, SEQ ID NO:80) is 321 amino acids long (Figure 32). The UNQ317 protein (SEQ ID NO:80) shown in Figure 32 has an estimated molecular weight of about 35,544 daltons and a pI of about 8.51. Analysis of the UNQ317 polypeptide as shown in Figure 32 evidences the presence of a glycosaminoglycan attachment site at about amino acid 149 to about amino acid 152 and a transmembrane domain from about amino acid 276 to about amino acid 306. A cDNA clone containing DNA45416 (SEQ ID NO:79) has been deposited with ATCC on February 5, 1998 and is assigned ATCC deposit no. 209620.

Q. Isolation of cDNA clones Encoding Human PRO363 (UNQ318)

Use of the ECD homology described above in a human fetal kidney library resulted in the isolation of the full-length DNA sequence DNA45419 (Fig. 33, SEQ ID NO:86) and the derived PRO363 native sequence protein UNQ318 (Fig. 34, SEQ ID NO:87).

The PCR primers (forward and reverse) and hybridization probe synthesized for use in the above procedure were:

forward PCR primer:

5'-CCAGTGCACAGCAGGCAACGAAGC-3'	(SEQ ID NO:88)

reverse PCR primer:

5'-ACTAGGCTGTATGCCTGGGTGGGC-3'	(SEQ ID NO:89)

hybridization probe:

5'-GTATGTACAAAGCATCGGCATGGTTGCAGGAGCAGTGACAGGC-3'	(SEQ ID NO:90)

Clone DNA45419 (SEQ ID NO:86) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 190-192 and ending at the stop codon (TGA) at nucleotide positions 1309-1311 (Figure 33), as indicated by bolded underline. The predicted PRO363 polypeptide precursor (*i.e.*, UNQ318, SEQ ID NO:87) is 373 amino acids long (Figure 34). The UNQ318 protein (SEQ ID NO:87) shown in Figure 34 has an estimated molecular weight of about 41,281 daltons and a pI of about 8.33. Analysis of the UNQ318 polypeptide as shown in Figure 34 evidences the presence of a transmembrane domain at about amino acid residue 221 to about residue 254. A cDNA clone containing DNA45419 (SEQ ID NO:86) has been deposited with ATCC on February 5, 1998 and is assigned ATCC deposit no. 209616.

R. Isolation of cDNA clones Encoding Human PRO364 (UNQ319)

Use of the ECD homology procedure described above in a human small intestine library resulted in the identification of an expressed sequence tag (EST) (Incyte EST No. 3003460) that encoded a polypeptide which showed homology to members of the tumor necrosis factor receptor (TNFR) family of polypeptides.

A consensus DNA sequence was then assembled relative to the Incyte 3003460 EST in a manner similar to that used in the ECD homology procedure which resulted in the isolation of the full-length DNA sequence DNA47365 (Fig. 35, SEQ ID NO:91) and the derived PRO364 native sequence protein UNQ319 (Fig. 36, SEQ ID NO:92).

The PCR primers (forward and reverse) and hybridization probes synthesized for use in the above-described screening procedure were:

forward PCR primer (44825.f1): 5'-CACAGCACGGGGCGATGGG-3' (SEQ ID NO:93)
forward PCR primer (44825.f2): 5'-GCTCTGCGTTCTGCTCTG-3' (SEQ ID NO:94)
forward PCR primer (44825.GITR.f):
5'-GGCACAGCACGGGGCGATGGGCGCGTTT-3' (SEQ ID NO:95)
reverse PCR primer (44825.r1): 5'-CTGGTCACTGCCACCTTCCTGCAC-3' (SEQ ID NO:96)
reverse PCR primer (44825.r2): 5'-CGCTGACCCAGGCTGAG-3' (SEQ ID NO:97)
reverse PCR primer (44825.GITR.r):
5'-GAAGGTCCCCGAGGCACAGTCGATACA-3' (SEQ ID NO:98)
hybridization probe (44825.p1):
5'-GAGGAGTGCTGTTCCGAGTGGGACTGCATGTGTGTCCAGC-3' (SEQ ID NO:99)
hybridization probe (44825.GITR.p):
5'-AGCCTGGGTCAGCGCCCCACCGGGGGTCCCGGGTGCGGCC-3' (SEQ ID NO:100)

Clone DNA47365 (SEQ ID NO:91) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 121-123 and ending at the stop codon (TGA) at nucleotide positions 844-846 (Figure 35). as indicated by bolded underline. The predicted PRO364 polypeptide precursor (*i.e.*, UNQ319, SEQ ID NO:92) is 241 amino acids long (Figure 36). The UNQ319 (SEQ ID NO:92) protein shown in Figure 36 has an estimated molecular weight of about 26,000 daltons and a pI of about 6.34. A potential N-glycosylation sites exists between amino acids 146 and 149 of the amino acid sequence shown in Figure 36. A putative signal sequence is from amino acids 1 to 25 and a potential transmembrane domain exists between amino acids 162 to 180 of the sequence shown in Figure 36. A cDNA clone containing DNA47365

(designated DNA47365-1206) has been deposited with ATCC on November 7, 1997 and is assigned ATCC Deposit No. ATCC 209436.

S. Isolation of cDNA clones Encoding Human PRO356 (UNQ313)(NL4)

An expressed sequence tag (EST) DNA database (LIFESEQ℠, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST (#2939340) was identified which showed homology to human TIE-2 L1 and TIE-2 L2.

Based on the EST, a pair of PCR primers (forward and reverse), and a probe were synthesized:

NL4,5-1: 5'-TTCAGCACCAAGGACAAGGACAATGACAACT-3' (SEQ ID NO: 103)
NL4,3-1: 5'-TGTGCACACTTGTCCAAGCAGTTGTCATTGTC-3' (SEQ ID NO: 104)
NL4,3-3: 5'-GTAGTACACTCCATTGAGGTTGG-3' (SEQ ID NO: 105).

Oligo dT primed cDNA libraries were prepared from uterus mRNA purchased from Clontech, Inc. (Palo Alto, CA, USA, catalog # 6537-1) in the vector pRK5D using reagents and protocols from Life Technologies, Gaithersburg, MD (Super Script Plasmid System). pRK5D is a cloning vector that has an sp6 transcription initiation site followed by an SfiI restriction enzyme site preceding the XhoI/NotI cDNA cloning sites. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized to greater than 1000 bp appropriately by gel electrophoresis, and cloned in a defined orientation into XhoI/NotI-cleaved pRK5D.

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO356 gene using the probe oligonucleotide and one of the PCR primers.

DNA sequencing of the clones isolated as described above gave a full-length DNA sequence encoding the native sequence PRO356 (NL4) (*i.e.*, DNA47470, SEQ ID NO:101) and the derived PRO356 protein sequence UNQ313 (SEQ ID NO:102).

The entire nucleotide sequence of DNA47470 is shown in Figure 37 (SEQ ID NO:101). Clone DNA47470 (SEQ ID NO:101) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 215-217, and a TAA stop codon at nucleotide positions 1038-1040, as indicated by bolded underline. The predicted PRO356 polypeptide is 346 amino acids long (*i.e.*, UNQ313 (SEQ ID NO:102), has a calculated molecular weight of 40,018 daltons and a pI of 8.19. A cDNA clone containing DNA47470 (SEQ ID NO:101) has been deposited with ATCC on October 28, 1997 and is assigned ATCC deposit no. 209422.

T. Isolation of cDNA clones Encoding Human PRO531 (UNQ332)

Use of the ECD homology procedure identified above in a human fetal brain library resulted in the isolation of the full-length DNA sequence DNA48314 (Fig. 39, SEQ ID NO:106) and the derived PRO531 native sequence protein UNQ332 (Fig. 40, SEQ ID NO:107).

The PCR primers (forward and reverse) and hybridization probe synthesized were:

forward PCR primer: 5'-CTGAGAACGCGCCTGAAACTGTG-3' (SEQ ID NO:108)
reverse PCR primer: 5'-AGCGTTGTCATTGACATCGGCG-3' (SEQ ID NO:109)

hybridization probe: (SEQ ID NO:110)

5'-TTAGTTGCTCCATTCAGGAGGATCTACCCTTCCTCCTGAAATCCGCGGAA-3'

Clone DNA48314 (SEQ ID NO:106) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 171-173 and ending at the stop codon (TGA) at nucleotide positions 2565-2567 (Figure 39), as indicated by bolded underline. The predicted PRO531 polypeptide precursor (*i.e.*, UNQ332, SEQ ID NO:107) is 789 amino acids long. The UNQ332 protein (SEQ ID NO:107) shown in Figure 39 has an estimated molecular weight of about 87552 daltons and a pI of about 4.84. A clone containing DNA48314 (SEQ ID NO:106) has been deposited with the ATCC on 26 March 1998, and has been assigned deposit number 209702.

Analysis of the UNQ332 amino acid sequence of SEQ ID NO:107 reveals a cadherin extracellular repeated domain signature at about amino acids 122-132, 231-241, 336-346, 439-449 and 549-559. An ATP/GTP-binding site motif A (P-loop) is found at about amino acids 285-292 of SEQ ID NO:107. N-, glycosylation sites are found at least at about amino acids 567-570, 786-790, 418-421 and 336-339, the signal peptide is at about amino acids 1-26, and the transmembrane domain is at about amino acids 685-712 of SEQ ID NO:107.

U. Isolation of cDNA clones Encoding Human PRO533 (UNQ334)

The EST sequence accession number AF007268, a murine fibroblast growth factor (FGF-15) was used to search various public EST databases (*e.g.*, GenBank, Dayhoff, *etc.*). The search was performed using the computer program BLAST or BLAST2 [Altschul *et al.*, *Methods in Enzymology*, 266:460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. The search resulted in the identification of GenBank EST AA220994, which has been identified as stratagene NT2 neuronal precursor 937230.

Based on this sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per *Ausubel et al., Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the PRO533 gene of interest by an *in vivo* cloning procedure using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal retina. The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents (*e.g.*, Invitrogen, San Diego, CA; Clontech, *etc.*) The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; Holmes *et al.*, *Science*, 253: 1278-1280 (1991)) in the unique XhoI and NotI sites.

A cDNA clone was sequenced in its entirety. The full length nucleotide sequence DNA49435 (SEQ ID NO:111) is shown in Figure 41. Clone DNA49435 (SEQ ID NO:111) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 464-466 and ending at the stop codon (TAA) at nucleotide positions 649-651, as indicated by bolded underline in Fig. 41. The predicted PRO533

polypeptide precursor (*i.e.*, UNQ334, SEQ ID NO:112) is 216 amino acids long, has a calculated molecular weight of 24,003 daltons and a pI of 6.99. Clone DNA49435-1219 has been deposited with ATCC (under the designation DNA49435-1219) on November 21, 1997 and is assigned ATCC deposit no. 209480.

The oligonucleotide sequences used in the above procedure were the following:

FGF15.f:	5'-ATCCGCCCAGATGGCTACAATGTGTA-3'	(SEQ ID NO:113)
FGF15.p:	5'-GCCTCCCGGTCTCCCTGAGCAGTGCCAAACAGCGGCAGTGTA-3'	(SEQ ID NO:114)
FGF15.r:	5'-CCAGTCCGGTGACAAGCCCAAA-3'	(SEQ ID NO:115)

V. Isolation of cDNA clones Encoding Human PRO1083 (UNQ540)

Use of the amylase yeast screen procedure described above on tissue isolated from human fetal kidney tissue resulted in an EST sequence which served as the template for the creation of the oligonucleotides below and screening as described above in a human fetal kidney library resulted in the isolation of the full length DNA sequence DNA50921 (Fig. 43, SEQ ID NO: 116) and the derived PRO1083 native sequence protein UNQ540 (SEQ ID NO:117).

The PCR primers (forward and reverse) and hybridization probes synthesized for use in the above procedure were the following:

forward primer: (43422.f1): 5'-GGCATTGGAGCAGTGCTGGGTG-3'	(SEQ ID NO:118)
forward primer: (43422.f2): 5'-AGAGCAACTCAGACAGCG-3'	(SEQ ID NO:119)
reverse primer: (43422.r1): 5'-TGGAGGCCTAGATGCGGCTGGACG-3'	(SEQ ID NO:120)
reverse primer: (43422.r2): 5'-CGAGGAGACCATCAGCAC-3'	(SEQ ID NO:121)
hybridization probe: (43422.p1): 5'-CCCAAACATCCTGCTTCTGCAACCACTTGACCTACTTTGCAGTGC-3'	(SEQ ID NO:122)

Clone DNA50921 (SEQ ID NO:116) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 154-156 and ending at the stop codon (TAG) at nucleotide positions 2233-2235 (Figure 43), as indicated by bolded underline. The predicted PRO1083 polypeptide precursor (*i.e.*, UNQ540, SEQ ID NO:117, Figure 44) is 693 amino acids long. The UNQ540 (SEQ ID NO:117) protein shown in Figure 44 has an estimated molecular weight of about 77738 and a pI of about 8.87. A clone containing DNA50921 has been deposited with the ATCC on May 12, 1998 and has been assigned deposit number 209859.

Analysis of the amino acid sequence UNQ540 (SEQ ID NO:117) reveals the putative signal peptide is at about amino acids 1-25, transmembrane domains are at about amino acids 382-398, 402-420, 445-468, 473-491, 519-537, 568-590 and 634-657, a microbodies C-terminal targeting signal at about amino acids 691-693, cAMP- and cGMP-dependent protein kinase phosphorylation sites at about amino acids 198-201 and 370-373, N-glycosylation sites at about amino acids 39-42, 148-151, 171-174, 234-237, 303-306. 324-227 and 341-344 and a G-protein coupled receptor family domain at about amino acids 475-504.

W. Isolation of cDNA clones Encoding Human PRO865 (UNQ434)

Use of the amylase yeast screen procedure described above on tissue isolated from human fetal kidney tissue resulted in an EST sequence which served as the template for the creation of the oligonucleotides below and screening as described above in a human fetal kidney library resulted in the isolation of the full length DNA sequence DNA53974 (Fig. 45, SEQ ID NO:123) and the derived PRO865 native sequence protein UNQ434 (SEQ ID NO:124).

The PCR primers (forward and reverse) and hybridization probes synthesized for use in the above procedure were the following:

forward primer: (48615.f1): 5'-AAGCTGCCGGAGCTGCAATG-3' (SEQ ID NO:125)
forward primer: (48615.f2): 5'-TTGCTTCTTAATCCTGAGCGC-3' (SEQ ID NO:126)
forward primer: (48615.f3): 5'-AAAGGAGGACTTTCGACTGC-3' (SEQ ID NO:127)
reverse primer: (48615.r1): 5'-AGAGATTCATCCACTGCTCCAAGTCG-3' (SEQ ID NO:128)
reverse primer: (48615.r2): 5'-TGTCCAGAAACAGGCACATATCAGC-3' (SEQ ID NO:129)
hybridization probe: (43422.p1): (SEQ ID NO:130)
5'-AGACAGCGGCACAGAGGTGCTTCTGCCAGGTTAGTGGTTACTTGGATGAT-3'

Clone DNA53974 (SEQ ID NO:123) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 173-175 and ending at the stop codon (TAA) at nucleotide positions 1577-1579 (Figure 45), as indicated by bolded underline. The predicted PRO865 polypeptide precursor (*i.e.*, UNQ865, SEQ ID NO:124) is 468 amino acids long. The UNQ434 (SEQ ID NO:124) protein shown in Figure 46 has an estimated molecular weight of about 54,393 and a pI of about 5.63. A clone containing DNA53974 (SEQ ID NO:123) has been deposited with the ATCC on April 14, 1998 and has been assigned deposit number 209774.

Analysis of the amino acid sequence UNQ434 (SEQ ID NO:124) reveals the putative signal peptide at about amino acid residues 1-23, potential N-glycosylation sites at about amino acids residue 280 and at about 384, a potential amidation site from about amino acid residue 94 to about residue 97, glycosaminoglycan attachment sites from about amino acid residue 20 to about 23 and from about residue 223 to about residue 226, an aminotransferase class-V pyridoxyl-phosphate amino acid sequence block from about amino acid residue 216 to about residue 222 and an amino acid sequence block similar to that found in the interleukin-7 protein from about amino acid residue 338 to about residue 343.

X. Isolation of cDNA clones Encoding Human PRO770 (UNQ408)

A public expressed sequence tag (EST) DNA database (Merck/Washington University) was searched with the full-length murine m-FIZZ1 DNA (DNA53517), and an EST, designated AA524300 was identified, which showed homology with the m-FIZZ1 DNA.

The full-length clone corresponding to the EST AA524300 was purchased from Incyte (Incyte Pharmaceuticals, Palo Alto, CA) and sequenced in entirety.

The entire nucleotide sequence of the resulting PRO770-encoding full-length clone is shown in Figure 47. This full-length clone, designated DNA54228 (SEQ ID NO:133), contains a single open reading frame with an apparent translation initiation site at nucleotide positions 100-102 (Fig.47; SEQ ID NO:133) and ending at the stop codon (TGA) at residues 433-435, as indicated by bolded underline. The predicted PRO770 polypeptide precursor (including a putative signal sequence of 20 amino acids) (*i.e.*, UNQ408, SEQ ID

NO:134) is 111 amino acids long, has a calculated molecular weight of 11,730 daltons and a pI of 7.82. Based upon its homology to m-FIZZ1 (50%, using the ALIGN software), the protein is believed to be the human homolog of m-FIZZ1, and has been designated h-FIZZ1. A cDNA clone containing DNA54228 (SEQ ID NO:133) has been deposited with ATCC and is assigned ATCC deposit no. 209801.

Identification and cloning of m-FIZZ1 (DNA53517)

Mouse asthma model Female Balb/C mice, 6 to 8 weeks of age, were separated into two experimental groups: controls and asthmatics. The asthmatic group was immunized intraperitoneally with 10 μg ovalbumin + 1 mg alum, while the control group was not. Two weeks later, mice were exposed daily to an aerosol of 10 mg/ml ovalbumin in PBS aerosolized with a UltraNeb nebulizer (DeVilbiss) at the rate of 2 ml/min for 30 min each day, for 7 consecutive days. One day after the last aerosol challenge, whole blood, serum and bronchoalveolar lavage (BAL) samples were collected and the lungs were harvested and preserved for histological examination, immuno-histochemistry and *in situ* hybridization.

*Gel electrophoresis of BAL samples*Examination of the BAL samples by gel electrophoresis on a 16% Tricine gel shows that a low molecular weight protein is expressed in the BAL samples from asthmatic mice but not in the BAL samples from control mice. This low molecular weight protein was termed m-FIZZ1 and was seen to co-migrate with a 8300 Dalton marker protein.

Partial protein sequence The protein of interest was transferred upon a PVDF membrane and sequenced by Edman degradation. This sequence served as a template for the preparation of various cloning oligos as described below.

Partial cDNA sequence We designed two degenerate oligonucleotide PCR primers corresponding to the putative DNA sequence for the first 7 and the last 7 amino acids of the partial protein sequence..

Oligo #1:

5'-ACA AAC GCG TGA YGA RAC NAT HGA RAT-3' (SEQ ID NO:135)

Oligo # 2:

5'-TGG TGC ATG CGG RTA RTT NGC NGG RTT-3' (SEQ ID NO:136)

cDNA prepared from the lungs of normal mice was used as a template for the PCR reaction which yielded an 88 bp product. This 88 bp product contained 54 known base pairs, encoding the PCR primers, and 34 novel base pairs, and encoded another partial mFIZZ-1 sequence.

Full length cDNA clone This second partial sequence was used to design primers which were ultimately successful in obtaining the full length FIZZ clone (DNA53517) by RT-PCR of mouse lung poly(A)$^+$ RNA.

Oligo #3:

5'-ACA AAC GCG TGC TGG AGA ATA AGG TCA AGG-3' (SEQ ID NO:137)

This oligo was used as an RT-PCR primer in combination with 5' and 3' amplimers from Clontech.

Oligo #4:

5'-ACT AAC GCG TAG GCT AAG GAA CTT CTT GCC-3' (SEQ ID NO: 138)

This oligo was used as an RT-PCR primer in combination with oligo d(T).

Y. Isolation of cDNA clones Encoding Human PRO769 (UNQ407)

A public expressed sequence tag (EST) DNA databases (Merck/Washington University) was searched with the full-length murine m-FIZZ1 DNA (DNA 53517) described above and the EST W42069 was identified.

The full-length clones corresponding to the EST fragment W42069 was obtained from Incyte Pharmaceuticals (Palo Alto, California), and sequenced in the entirety, which ultimately resulted in the identification of the full length nucleotide sequence DNA54231 (SEQ ID NO:139).

The nucleotide sequence corresponding to the full length, native sequence PRO769 clone is shown in Figure 49. This clone, designated DNA 54231 (SEQ ID NO:139) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 75-77 and ending at the stop codon (TGA) at residues 417-419, as indicated by bolded underline (Fig. 49). The predicted PRO769 polypeptide precursor (including a signal sequence of 10 amino acids)(*i.e.*, UNQ407, SEQ ID NO:140) is 114 amino acids long, has a calculated molecular weight of 12,492 daltons and a pI of 8.19. Based on its homology to m-FIZZ1 (34%, using the ALIGN software) the protein was designated m-FIZZ3. A clone containing DNA54231 (designated DNA54231-1366) has been deposited with ATCC on April 23, 1998 and has been assigned ATCC deposit no. 209802.

Z. Isolation of cDNA clones Encoding Human PRO788 (UNQ430)

Use of the ECD homology procedure identified above resulted in the identification of the partial length EST sequence 2777282. Further analysis of the corresponding full-length sequence resulted in the identification of DNA56405 (SEQ ID NO:141) and the derived native sequence PRO788 protein UNQ430 (SEQ ID NO:142).

Clone DNA56405 (SEQ ID NO:141) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 84-86 and ending at the stop codon (TAG) at nucleotide positions 459-461 (Figure 51), as indicated by bolded underline. The predicted native sequence PRO788 polypeptide precursor (*i.e.*, UNQ430, SEQ ID NO:142) is 125 amino acids long (Figure 52), has a calculated molecular weight of 13,115 daltons and a pI of 5.90. The UNQ430 (SEQ ID NO:142) protein shown in Figure 52 has an estimated molecular weight of about 13115 and a pI of about 5.90. A clone containing DNA56405 (SEQ ID NO:142) has been deposited with the ATCC on May 6, 1998 and has been assigned deposit number209849. In the event of a discrepancy in the nucleotide sequence of the deposit and the sequences disclosed herein, it is understood that the deposited clone contains the correct sequence. It is further understood that the methodology of sequencing for the sequences provided herein are based on known sequencing techniques.

Analysis of UNQ430 (SEQ ID NO:52) shown in Figure 52 reveals a signal peptide at about amino acids 1-17 and an N-glycosylation site is at about amino acids 46.

AA. Isolation of cDNA clones Encoding Human PRO1114 (UNQ557)

Use of the amylase yeast screen procedure described above on tissue isolated from human fetal kidney tissue resulted in an EST sequence which served as the template for the creation of the oligonucleotides below and screening as described above in a human breast carcinoma library resulted in the

isolation of the full length DNA sequence DNA57033 (Fig. 53, SEQ ID NO:143) and the derived PRO1114 native sequence protein UNQ557 (Fig. 54, SEQ ID NO:144).

The PCR primers used in the isolation screen described in the previous paragraph were:

forward primer: (48466.f1): 5'-AGGCTTCGCTGCGACTAGACCTC-3' (SEQ ID NO:145)

reverse primer: (48466.r1): 5'-CCAGGTCGGGTAAGGATGGTTGAG-3' (SEQ ID NO:146)

hybridization probe: 48466.p1):

5'-TTTCTACGCATTGATTCCATGTTTGCTCACAGATGAAGTGGCCATTCTGC-3' (SEQ ID NO:147)

Clone DNA57033 (SEQ ID NO:143) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 250-252 and ending at the stop codon (TAG) found at nucleotide positions 1183-1185 (Figure 53, SEQ ID NO:143), as indicated by bolded underline. The predicted PRO1114 polypeptide precursor (*i.e.*, UNQ557, SEQ ID NO:144) is 311 amino acids long, has a calculated molecular weight of approximately 35,076 daltons and an estimated pI of approximately 5.04. Analysis of the full-length PRO1114 sequence shown in Figure 54 (SEQ ID NO:144) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 29, a transmembrane domain from about amino acid 230 to about amino acid 255, potential N-glycosylation sites from about amino acid 40 to about amino acid 43 and from about amino acid 134 to about amino acid 137, an amino acid sequence block having homology to tissue factor proteins from about amino acid 92 to about amino acid 119 and an amino acid sequence block having homology to integrin alpha chain proteins from about amino acid 232 to about amino acid 262. A cDNA clone containing DNA57033 (SEQ ID NO:143) has been deposited with ATCC on May 27, 1998 and is assigned ATCC deposit no. 209905.

AB. Isolation of cDNA clones Encoding Human PRO1007 (UNQ491)

Use of the ECD homology procedure described above resulted in the identification of an EST sequence designated Merck EST T70513, which was derived from human liver tissue (clone 83012 from library 341) was further examined. The corresponding full-length clone was further examined and sequenced, resulting in the isolation of the full-length DNA sequence DNA57690 (Fig. 55, SEQ ID NO:145) and the derived PRO1007 native sequence protein UNQ491 (Fig. 56, SEQ ID NO:146).

Clone DNA57690 (SEQ ID NO:145) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 16-18 and ending at the stop codon (TGA) at nucleotide positions 1054-1056 (Figure 55), as indicated by bolded underline. The predicted PRO1007 polypeptide precursor (*i.e.*, UNQ491, SEQ ID NO:146) is 346 amino acids long (Figure 56), has a calculated molecular weight of 35,971 daltons and a pI of 8.17. The UNQ491 (SEQ ID NO:146) protein shown in Figure 56 has an estimated molecular weight of about 35971 daltons and a pI of about 8.17. A cDNA clone containing DNA57690 (SEQ ID NO:145) has been deposited with the ATCC on 9 June 1998, and has been assigned deposit number 209950.

Analysis of the amino acid sequence of UNQ491 (SEQ ID NO:146) reveals the putative signal peptide at about amino acid residues 1-30, a transmembrane domain at about amino acid residues 325-346, N-glycosylation sites at about amino acid residues 118, 129, 163, 176, 183 and 227 and a Ly-6/u-Par domain proteins at about amino acid residues 17-36 and 209-222. The corresponding nucleotides of the amino acids presented herein can be routinely determined given the sequences provided herein.

AC. Isolation of cDNA clones Encoding Human PRO1184 (UNQ598)

Use of the signal algorithm procedure described above resulted in the identification of Incyte EST 1428374 which was derived from an ileum tissue library (39, SINTBST01). Further examination of the full-length clone corresponding to this sequence resulted in the isolation of the full-length DNA59220 (Fig. 57, SEQ ID NO:147) and the derived PRO1184 native sequence protein UNQ598 (Fig. 58, SEQ ID NO:148).

UNQ598 (SEQ ID NO:148), as shown in Figure 58 exhibits an apparent translation initiation site at nucleotide positions 106-108 and ending at the stop codon (TGA) found at nucleotide positions 532-534, as indicated by bolded underline. The predicted PRO1184 polypeptide precursor (*i.e.*, UNQ598, SEQ ID NO:148) is 142 amino acids long, has a calculated molecular weight of approximately 15690 daltons and an estimated pI of approximately 9.64. Analysis of UNQ598 (SEQ ID NO:148) evidences the presence of a signal peptide at about amino acids 1-38. A cDNA clone containing DNA59220 (SEQ ID NO:147) has been deposited with the ATCC on 9 June 1998, and has been assigned deposit number 209962. It is understood that the deposited clone has the actual sequences and that representations are presented herein.

AD. Isolation of cDNA clones Encoding Human PRO1031 (UNQ516)

Use of the ECD homology procedure described above resulted in the identification of the EST sequence Merck W74558 (clone 344649). The corresponding full-length clone was examined and sequenced resulting in the isolation of DNA sequencing gave the full-length DNA sequence DNA59294 (Fig. 59, SEQ ID NO:149) and the derived PRO1031 native sequence protein UNQ516 (Fig. 60, SEQ ID NO:150).

Clone DNA59294 (SEQ ID NO:149) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 42-44 and ending at the stop codon (TGA) at nucleotide positions 582-584 (Figure 59), as indicated by bolded underline. The predicted PRO1031 polypeptide precursor (*i.e.*, UNQ516, SEQ ID NO:150) is 180 amino acids long (Figure 60). The UNQ516 protein shown in Figure 60 has an estimated molecular weight of about 20437 and a pI of about 9.58. Clone DNA59294 (SEQ ID NO:149) has been deposited with the ATCC on May 14, 1998 and has been assigned deposit number 209866. Regarding the sequence, it is understood that the deposited clone contains the correct sequence, and the sequences provided herein are based on known sequencing techniques.

Analysis of the amino acid sequence of UNQ516 (SEQ ID NO:150) reveals the putative signal peptide at about amino acid residues 1-20, an N-glycosylation site is at about amino acid residue 75. A region having sequence identity with IL-17 is at about amino acid residues 96-180. The corresponding nucleotides can be routinely determined given the sequences provided herein.

AE. Isolation of cDNA clones Encoding Human PRO1346 (UNQ701)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the isolation of the full-length DNA sequence DNA59776 (Fig. 61, SEQ ID NO:151) and the derived PRO1346 native sequence protein UNQ701 (Fig. 62, SEQ ID NO:152).

The PCR primers (forward and reverse) and hybridization probe used in the isolation of DNA59776 (SEQ ID NO:151) were the following:

forward PCR primer (45668.f1): 5'-CACACGTCCAACCTCAATGGGCAG-3' (SEQ ID NO:153)

reverse PCR primer (45668.r1): 5'-GACCAGCAGGGCCAAGGACAAGG-3' (SEQ ID NO:154)
hybridization probe (45668.p1): (SEQ ID NO:155)
5'-GTTCTCTGAGATGAAGATCCGGCCGGTCCGGGAGTACCGCTTAG-3'

Clone DNA59776 (SEQ ID NO:151) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 1-3 (ATG), and an apparent stop codon (TAG) at nucleotide positions 1384-1386 (TAG). The predicted PRO1346 polypeptide precursor (*i.e.*, UNQ701, SEQ ID NO:152) is 461 amino acids long. The protein contains an apparent type II transmembrane domain at amino acid positions from about 31 to about 50, fibrinogen beta and gamma chains C-terminal domain signature at about amino acid positions 409-421 and a leucine zipper patterns at about amino acid positions 140-161, 147-168, 154-175 and 161-182.

A cDNA clone containing DNA59776, designated as DNA59776-1600, has been deposited with ATCC on August 18, 1998 and is assigned ATCC deposit no. 203128. The UNQ701 (SEQ ID NO:152) protein shown in Figure 62 has an estimated molecular weight of about 50744 daltons and a pI of about 6.38.

AF. Isolation of cDNA clones Encoding Human PRO1155 (UNQ585)

Use of the signal algorithm procedure described above resulted in the identification of Incyte EST 2858870 which was derived from an ileum tissue library (39, SININOT03). Further examination of the full-length clone corresponding to this sequence resulted in the isolation of the full-length DNA sequence DNA59849 (Fig. 63, SEQ ID NO:156) and the derived PRO1155 native sequence protein UNQ585 (Fig. 64, SEQ ID NO:157).

The UNQ585 (SEQ ID NO:157) polypeptide shown in Figure 64 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 158-160 and ending at the stop codon (TAA) found at nucleotide positions 563-565, as indicated by bolded underline. The predicted PRO1155 polypeptide precursor (*i.e.*, UNQ585, SEQ ID NO:157) is 135 amino acids long, and signal peptide appears at about amino acids residues 1 to about 18, a leucine zipper pattern appears at about amino acid residues 43 to 64 and a tachykinin family signature appears at about amino acid residues 86 to about 91. UNQ585 (SEQ ID NO:157) has a calculated molecular weight of approximately 14833 daltons and an estimated pI of approximately 9.78. A cDNA clone containing DNA59849 (SEQ ID NO:156), designated as DNA59849-1504, has been deposited with ATCC on June 16, 1998 and is assigned ATCC deposit no. 209986.

AG. Isolation of cDNA clones Encoding Human PRO1250 (UNQ633)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence from the Incyte database, designated Incyte EST cluster sequence no. 56523. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST 3371784. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA60775 (Fig. 65, SEQ ID NO:158) and the derived PRO1250 native sequence protein UNQ633 (Fig. 66, SEQ ID NO:159).

Clone DNA60775 (SEQ ID NO:158) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 74-76 and ending at the stop codon (TAG) at nucleotide

positions 2291-2293 (Figure 65). The predicted PRO1250 polypeptide precursor (*i.e.*, UNQ633, SEQ ID NO:159) is 739 amino acids long (Figure 66). The UNQ633 (SEQ ID NO:159) protein shown in Figure 66 has an estimated molecular weight of about 82,263 daltons and a pI of about 7.55. Analysis of UNQ633 (SEQ ID NO:159) evidences the presence of the following: a type II transmembrane domain from about amino acid residues 61 to about 80, a putative AMP-binding domain signature sequence from about amino acid residue 314 to about 325, and potential N-glycosylation sites from about amino acid residues 102 to about 105, from about amino acid residues 588 to about 591 and from about amino acid residues 619 to about 622. A cDNA clone containing DNA60775 (SEQ ID NO:158) has been deposited with the ATCC on September 1, 1998 and is assigned ATCC deposit no. 203173.

AH. Isolation of cDNA clones Encoding Human PRO1312 (UNQ678)

An EST (DNA55773) was identified in a human fetal kidney cDNA library using a yeast screen, that preferentially represents the 5' ends of the primary cDNA clones. Based on the DNA55773 sequence, oligonucleotides were synthesized for use as probes to isolate the full-length DNA sequence DNA61873 (Fig. 67, SEQ ID NO:160) and the derived PRO1312 native sequence UNQ678 (SEQ ID NO:161).

The full length DNA61873 clone shown in Figures 67 (SEQ ID NO:160) contains a single open reading frame with an apparent translation initiation site at about nucleotide positions 7-9 and ending at the stop codon (TGA) found at about nucleotide positions 643-645, as indicated by bolded underline. The predicted PRO1312 polypeptide precursor (*i.e.*, UNQ678, SEQ ID NO:161) is 212 amino acids long. UNQ678 (SEQ ID NO:161) has a calculated molecular weight of approximately 24,024 daltons and an estimated pI of approximately 6.26. Other features include a signal peptide at about amino acids 1-14; a transmembrane domain at about amino acids 141-160, and potential N-glycosylation sites at about amino acids 76-79 and 93-96. A clone containing DNA61873 (SEQ ID NO:160) has been deposited with the ATCC on August 18, 1998, under the designation DNA61873-1312, and has been assigned deposit number 203132.

AI. Isolation of cDNA clones Encoding Human PRO1192 (UNQ606)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the isolation of the full-length DNA sequence DNA62814 (Fig. 69, SEQ ID NO:162) and the derived PRO1192 native sequence protein UNQ606 (Fig. 70, SEQ ID NO:163).

The PCR primers (forward and reverse) and hybridization probe used in the isolation of DNA62814 (SEQ ID NO:162) were the following:

forward PCR primer (35924.f1): 5'-CCGAGGCCATCTAGAGGCCAGAGC-3' (SEQ ID NO:164)
reverse PCR primer (35924.r1): 5'-ACAGGCAGAGCCAATGGCCAGAGC-3' (SEQ ID NO:165).
hybridization probe (35924.p1): (SEQ ID NO:166).
5'-GAGAGGACTGCGGGAGTTTGGGACCTTTGTGCAGACGTGCTCATG-3'

Clone DNA62814 (Fig. 69, SEQ ID NO:162) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 121-123, and an apparent stop codon (TAA) at nucleotide positions 766-768, as indicated by bolded underline. The predicted PRO1192 polypeptide precursor (*i.e.*, UNQ606, SEQ ID NO:163) is 215 amino acids long. The UNQ606 (SEQ ID NO:163) polypeptide precursor shown in Figure 70 has a signal peptide at about amino acids 1-21; a transmembrane domain at about amino

acids 153-176; potential N-glycosylation sites at about amino acids 39-42 and 118-121; and homology with myelin P0 proteins at about amino acids 27-68 and 99-128. The UNQ606 (SEQ ID NO:163) shown in Figure 70 has an estimated molecular weight of about 24,484 Daltons and a pI of about 6.98.

A cDNA clone containing DNA62814 (SEQ ID NO:162), designated as DNA62814-1521, was deposited with the ATCC on August 4, 1998, and is assigned ATCC deposit no. 203093.

AJ. Isolation of cDNA clones Encoding Human PRO1246 (UNQ630)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence from the Incyte database, designated Incyte EST cluster sequence no. 56853. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST 2481345. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA64885 (Fig. 71, SEQ ID NO:167) and the derived PRO1246 native sequence protein UNQ630 (Fig. 72, SEQ ID NO:168).

Clone DNA64885 (SEQ ID NO:167) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 119-121 and ending at the stop codon (TGA) at nucleotide positions 1727-1729 (Figure 71), as indicated by bolded underline. The predicted PRO1246 polypeptide precursor (*i.e.*, UNQ630, SEQ ID NO:168) is 536 amino acids long (Figure 72), has an estimated molecular weight of about 61,450 daltons and a pI of about 9.17. Analysis of UNQ630 (Fig. 72, SEQ ID NO:168) reveals the following: a signal peptide from about amino acid 1 to about amino acid 15, potential N-glycosylation sites from about amino acid 108 to about amino acid 111, from about amino acid 166 to about amino acid 169, from about amino acid 193 to about amino acid 196, from about amino acid 262 to about amino acid 265, from about amino acid 375 to about amino acid 378, from about amino acid 413 to about amino acid 416 and from about amino acid 498 to about amino acid 501 and amino acid sequence blocks having homology to sulfatase proteins from about amino acid 286 to about amino acid 315, from about amino acid 359 to about amino acid 369 and from about amino acid 78 to about amino acid 97. A cDNA containing DNA64885 (SEQ ID NO:167), designated DNA64885-1529, has been deposited with ATCC on November 3, 1998 and is assigned ATCC deposit no. 203457.

AK. Isolation of cDNA clones Encoding Human PRO1283 (UNQ653)

Use of the ECD homology procedure described above in a human breast tumor tissue library resulted in the isolation of the full-length DNA sequence DNA65404 (Fig. 73, SEQ ID NO:169) and the derived PRO1283 native sequence protein UNQ653 (Fig. 74, SEQ ID NO:170).

The PCR primers (forward and reverse) and hybridization probes used in the isolation of DNA65404 (SEQ ID NO:169) were the following:

forward PCR primer (28753.f1):	5'-GGAGATGAAGACCCTGTTCCTG-3'	(SEQ ID NO:171)
forward PCR primer (28753.f11):	5'-GGAGATGAAGACCCTGTTCCTGGGTG-3'	(SEQ ID NO:172)
reverse PCR primer (28753.r1):	5'-GTCCTCCGGAAAGTCCTTATC-3'	(SEQ ID NO:173)
reverse PCR primer (28753.r11):	5'-GCCTAGTGTTCGGGAACGCAGCTTC-3'	(SEQ ID NO:174)
hybridization probe (28753.p1):		(SEQ ID NO:175)

5'-CAGGGACCTGGTACGTGAAGGCCATGGTGGTCGATAAGGACTTTCCGGAG-3'

hybridization probe (28753.p11): (SEQ ID NO:176)

5'-CTGTCCTTCACCCTGGAGGAGGAGGATATCACAGGGACCTGGTAC-3'

Clone DNA65404 (SEQ ID NO:169) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 45-47 and ending at the stop codon (TAG) at nucleotide positions 555-557 (Figure 73), as indicated by bolded underline. The predicted PRO1283 polypeptide precursor (*i.e.*, UNQ653, SEQ ID NO:170) is 170 amino acids long (Figure 74). The UNQ653 (SEQ ID NO:170) protein shown in Figure 74 has an estimated molecular weight of about 19,457 daltons and a pI of about 9.10. Analysis of the UNQ653 (SEQ ID NO:170) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 17. A cDNA clone containing DNA65404 (SEQ ID NO:169), designated DNA65404-1551, has been deposited with ATCC on September 9, 1998 and is assigned ATCC deposit no. 203244.

AL. Isolation of cDNA clones Encoding Human PRO1195 (UNQ608)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 32204 from the Incyte database. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST352980. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA65412 (Fig. 75, SEQ ID NO:177) and the derived PRO1195 native sequence protein UNQ608 (Fig. 76, SEQ ID NO:178).

The full length clone DNA65412 (SEQ ID NO:177) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 58-60 and ending at the stop codon (TAG) found at nucleotide positions 511-513 (Figure 75), as indicate by bolded underline. The predicted PRO1195 polypeptide precursor (*i.e.*, UNQ608, Figure 76, SEQ ID NO:178) is 151 amino acids long, has a calculated molecular weight of 17.227 daltons and a pI of 5.33. Analysis of UNQ608 (SEQ ID NO:178) reveals a signal sequence at about amino acids 1-22, a calculated molecular weight of approximately 17277 daltons and an estimated pI of approximately 5.33. A cDNA clone containing DNA65412 (SEQ ID NO:177), designated as DNA65412-1523, was deposited with the ATCC on August 4, 1998 and is assigned ATCC deposit no. 203094.

AM. Isolation of cDNA clones Encoding Human PRO1343 (UNQ698)

Use of the amylase yeast screen procedure described above on tissue isolated from human smooth muscle cell tissue resulted in an EST sequence which served as the template for the creation of the oligonucleotides below and screening as described above in a human smooth muscle cell tissue library resulted in the isolation of the full length DNA sequence DNA66675 (Fig. 77, SEQ ID NO:179) and the derived PRO1343 native sequence protein UNQ698 (Fig. 78, SEQ ID NO:180).

The oligonucleotide probes employed were as follows:

forward PCR primer (48921.f1) 5'-CAATATGCATCTTGCACGTCTGG-3' (SEQ ID NO:181)

reverse PCR primer (48921.r1) 5'-AAGCTTCTCTGCTTCCTTTCCTGC-3' (SEQ ID NO:182)

hybridization probe (48921.p1)

5'-TGACCCCATTGAGAAGGTCATTGAAGGGATCAACCGAGGGCTG-3' (SEQ ID NO:183)

The full length clone DNA66675 (SEQ ID NO:179) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 71-73, and a stop signal (TAA) at nucleotide positions 812-814 (Figure 77), as indicated by bolded underline. The predicted PRO1343 polypeptide precursor (*i.e.*, UNQ698, SEQ ID NO:180, Fig. 78) is 247 amino acids long, has a calculated molecular weight of approximately 25,335 daltons and an estimated pI of approximately 7.0. Analysis of the UNQ698 sequence shown in Figure 78 (SEQ ID NO:180) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 25 and a homologous region to circumsporozoite repeats from about amino acid 35 to about amino acid 225. A cDNA clone containing DNA66675 (SEQ ID NO:179), designated DNA66675-1587, has been deposited with ATCC on September 22, 1998 and is assigned ATCC deposit no. 203282.

Alternatively, a comparison of the yeast EST sequence isolated from the amylase screen above was screened against various EST databases, both public and private (*e.g.*, see ECD homology procedure, above) resulting in the identification of Incyte EST clone no. 4701148. Further analysis and sequencing of the corresponding full-length clone resulted in isolation of the DNA66675 sequence (SEQ ID NO:179) shown in Figure 77.

AN. Isolation of cDNA clones Encoding Human PRO1418 (UNQ732)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 10698 (Incyte cluster 121480). This sequence was then compared to a variety of various EST databases (including those derived from a placenta tissue library) as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST1306026. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA68864 (Fig. 79, SEQ ID NO:184) and the derived PRO1418 native sequence protein UNQ732 (Fig. 80, SEQ ID NO:185).

The full length clone shown in Figure 79 (DNA68864, SEQ ID NO:184) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 138-140 and ending at the stop codon (TAA) found at nucleotide positions 1188-1190, as indicated by bolded underline. The predicted PRO1418 polypeptide precursor (*i.e.*, UNQ732, SEQ ID NO:185) is 350 amino acids long with a signal peptide at about amino acids 1-19, a calculated molecular weight of approximately 39003 daltons and an estimated pI of approximately 5.59. A cDNA clone containing DNA68864 (SEQ ID NO:184), designated as DNA68864-1629 was deposited with the ATCC on September 22, 1998 and is assigned ATCC deposit no. 203276.

AO. Isolation of cDNA clones Encoding Human PRO1387 (UNQ722)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 10298. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST3507924. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA68872 (Fig. 81, SEQ ID NO:186) and the derived PRO1387 native sequence protein UNQ722 (Fig. 82, SEQ ID NO:187).

Clone DNA68872 (SEQ ID NO:186) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 76-78 and ending at the stop codon (TGA) at nucleotide positions 1258-1260 (Figure 81), as indicated by bolded underline. The predicted PRO1387 polypeptide precursor (*i.e.*, UNQ722, SEQ ID NO:187) is 394 amino acids long. The UNQ722 (SEQ ID NO:187) protein shown in Figure 82 has an estimated molecular weight of about 44,339 daltons and a pI of about 7.10. UNQ722 (SEQ ID NO:187) further contains a signal peptide from about amino acid residues 1 to about residue 19, a transmembrane domain from about residue 275 to about residue 296, potential N-glycosylation sites at about residues 76, 231, 302, 307 and 376 and amino acid sequence blocks having homology to myelin p0 protein from about amino acid residue 210 to about residue 239 and from about amino acid residue 92 to about residue 121. A cDNA clone containing DNA68872, designated as DNA68872-1620, has been deposited with the ATCC on August 25, 1998 and is assigned ATCC deposit no. 203160.

AP. Isolation of cDNA clones Encoding Human PRO1410 (UNQ728)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 98502. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST1257046. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA68874 (Fig. 83, SEQ ID NO:188) and the derived PRO1387 native sequence protein UNQ728 (Fig. 84, SEQ ID NO:189).

Clone DNA68874 (SEQ ID NO:188) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 152-154 and ending at the stop codon (TGA) at nucleotide positions 866-868 (Figure 83), as indicated by bolded underline. The predicted PRO1410 polypeptide precursor (*i.e.*, UNQ728, SEQ ID NO:189) is 238 amino acids long (Figure 84). The UNQ728 protein (SEQ ID NO:189) shown in Figure 84 has an estimated molecular weight of about 25,262 daltons and a pI of about 6.44, a signal peptide from about amino acid residue 1 to about residue 20, a transmembrane domain from about amino acid residue 194 to about residue 220 and a potential N-glycosylation site at about amino acid residue 132. A clone containing DNA68874 (SEQ ID NO:188) has been deposited with ATCC on September 22, 1998 and is assigned ATCC deposit no. 203277.

AQ. Isolation of cDNA clones Encoding Human PRO1917 (UNQ900)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence 85496. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST3255033. This EST was derived from an ovarian tumor library. Further examination and sequencing of the full-length clone corresponding to this EST sequence resulted in the isolation of the full-length DNA sequence DNA76400 (Fig. 85, SEQ ID NO:190) and the derived PRO1917 native sequence protein UNQ900 (Fig. 86, SEQ ID NO:191).

The full length clone DNA76400 (SEQ ID NO:190) shown in Figure 85 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 6 to 9 and ending at the stop codon (TGA) found at nucleotide positions 1467 to 1469 as indicated by bolded underline. The predicted PRO1917 polypeptide precursor (*i.e.*, UNQ900, SEQ ID NO:191) is 487 amino acids long. UNQ900 (SEQ ID

NO:191) has a calculated molecular weight of approximately 55,051 daltons and an estimated pI of approximately 8.14. Additional features include: a signal peptide at about amino acid residues 1-30; potential N-glycosylation sites at about amino acid residues 242 and 481, protein kinase C phosphorylation sites at about amino acid residues 95-97, 182-184, and 427-429; N-myristoylation sites at about amino acid residues 107-112, 113-118, 117-122, 118-123, and 128-133; and an endoplasmic reticulum targeting sequence at about amino acid residues 484-487.

AR. Isolation of cDNA clones Encoding Human PRO1868 (UNQ859)

Use of the ECD homology procedure described above in a human fetal liver library resulted in the identification of EST clone no. 2994689. Further analysis and sequencing of the corresponding full-length clone resulted in the isolation of DNA77624 (Fig. 87, SEQ ID NO:192) and the derived PRO1868 native sequence protein UNQ859 (Fig. 88, SEQ ID NO:193).

Clone DNA77624 (Fig. 88, SEQ ID NO:193) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 51-53 and ending at the stop codon (TGA) at nucleotide positions 981-983, as indicated by bolded underline. The predicted PRO1868 polypeptide precursor (*i.e.*, UNQ859, SEQ ID NO:193, Fig. 89) is 310 amino acids long. The UNQ859 (SEQ ID NO:193) protein shown in Figure 89 has an estimated molecular weight of about 35,020 daltons and a pI of about 7.90, a transmembrane domain from about amino acid residue 243 to about residue 263, potential N-glycosylation sites at about amino acid residues 104 and 192, a cAMP- and cGMP-dependent protein kinase phosphorylation site from about amino acid residues 107 to about residue 110, casein kinase II phosphorylation sites from about amino acid residues 106 to about residue 109 and from about amino acid residue 296 to about residue 299, a tyrosine kinase phosphorylation site from about amino acid residue 69 to about residue 77 and potential N-myristolation sites from about amino acid residue 26 to about residue 31, from about residue 215 to about residue 220, from about residue 226 to about residue 231, from about residue 243 to about residue 248, from about residue 244 to about residue 249 and from about residue 262 to about residue 267. A cDNA clone containing DNA77624 (SEQ ID NO:193) has been deposited with ATCC on December 22, 1998 and is assigned ATCC deposit no 203553.

AS. Isolation of cDNA clones Encoding Human PRO205 (UNQ179)

Use of the ECD procedure above resulted in the identification of an EST sequence derived from a human retinal library. Additional effort to identify the full length clone using an *in vitro* cloning procedure were unable to identify another PRO205 encoding DNA sequence.

DNA sequence encoding other polypeptide of substantial homology to the UNQ179 (SEQ ID NO:229) polypeptide of Figure 90 may be found as GenBank submissions AB033089_1 and HSM802147_1.

Clone DNA30868 (SEQ ID NO:89) contains what is believed to be an incomplete open reading frame with an apparent translation initiation site at nucleotide positions 405-407 as indicated by bolded underline in Figure 89. The predicted partial length PRO1868 polypeptide precursor (*i.e.*, UNQ179, SEQ ID NO:229) is 343 amino acids long, has a calculated molecular weight of 39285 daltons and a pI of 6.06.

Analysis of the UNQ179 (SEQ ID NO:229) shown in Figure 90 reveals a signal peptide at about amino acid residues 1 to 20, an N-glycosylation site at about amino acid residues 318-322, tyrosine kinase phosphorylation sites at about amino acids residues 21-29 and 211-220, N-myristolation sites at about residues 63-69, 83-89 and 317-323 and a prokaryotic membrane lipoprotein lipid attachment site at about residues 260-271. A cDNA clone containing DNA30868 (SEQ ID NO:228) has been deposited with the ATCC on March 2, 2000 under the designation DNA30868-1156 and has been assigned ATCC deposit no. _____.

AT. Isolation of cDNA clones Encoding Murine PRO21 (UNQ21)

The isolation of DNA36638 (Fig. 91, SEQ ID NO:230), which encodes the native sequence PRO21 polypeptide UNQ21 (Fig. 92, SEQ ID NO:231) has been previously described in U.S.P. 5,955,420. Additional cloning and characterizing information can be found in Schneider *et al., Cell* 54 (6): 787-93 (1988) and in Manfioletti *et al., Mol Cell Biol.* 13 (8): 4976-85 (1993).

Clone DNA36638 contains a single open reading frame with an apparent translation initiation site at nucleotide residues 168-170 and ending at the stop codon (TAG) at nucleotide residues 2187-2189 (Figure 91), as indicated by bolded underline. The predicted PRO21 polypeptide precursor (*i.e.*, UNQ21, SEQ ID NO:231) is 673 amino acids long, has a calculated molecular weight of 74,512 daltons and a pI of 5.45. A cDNA clone containing DNA36638 has been deposited with the ATCC under the designation DNA36638-1056 on November 12, 1997 and has been assigned ATCC deposit number 209456.

Analysis of the UNQ21 polypeptide of Figure 92 (SEQ ID NO:231) reveals a signal sequence at about amino acid residues 1-27, a transmembrane domain at about amino acid residues 619-635, N-glycosylation sites at about residues 417-421 and 488-492, N-myristolation sites at about amino acid residues 126-132, 135-141, 146-152, 173-179, 214-220, 253-259, 346-352, 374-380, 440-446, 479-485, 497-503, 517-523, 612-618, aspartic acid and asparagine hydroxylation sites at about amino acid residues 130-142, 168-180, 209-221 and 248-260, a vitamin K-dependent carboxylation domain and an EGF-like domain cysteine pattern signature at about amino acid residues 139-151.

AU. Isolation of cDNA clones Encoding Human PRO269 (UNQ236)

Use of the ECD homology procedure described above in a human fetal kidney library in combination with an *in vitro* cloning procedure using the probe oligonucleotide and one of the primer pairs below resulted in the identification of the full length DNA sequence DNA38260 (Fig. 93, SEQ ID NO:232) and the derived PRO269 native sequence protein UNQ236 (Fig. 94, SEQ ID NO:233).

The forward and reverse PCR primers and the hybridization probe used were the following:

forward PCR primer (.f1): (SEQ ID NO:234)
5'-TGGAAGGAGATGCGATGCCACCTG -3'

forward PCR primer (.f2): (SEQ ID NO:235)
5'-TGACCAGTGGGGAAGGACAG-3'

forward PCR primer (.f3): (SEQ ID NO:236)
5'-ACAGAGCAGAGGGTGCCTTG-3'

reverse PCR primer (.r1): (SEQ ID NO:237)
5'-TCAGGGACAAGTGGTGTCTCTCCC-3'

reverse PCR primer(.r2): (SEQ ID NO:238)
5'-TCAGGGAAGGAGTGTGCAGTTCTG-3'
hybridization probe: (SEQ ID NO:239)
5'-ACAGCTCCCGATCTCAGTTACTTGCATCGCGGACGAAATCGGCGCTCGCT-3'

Clone DNA38260 (SEQ ID NO:232) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 314-316 and ending at the stop codon (TAG) at nucleotide positions 1784-1786 (Fig. 93), as indicated by bolded underline. The predicted PRO269 polypeptide precursor is 490 amino acids long (*i.e.*, UNQ236, Fig. 94, SEQ ID NO:233), has a calculated molecular weight of 51,636 daltons and a pI of 6.29. A cDNA clone containing DNA38260 (SEQ ID NO:232) has been deposited with ATCC on October 17, 1997 and is assigned ATCC deposit no. 209397.

Analysis of the UNQ236 polypeptide of Figure 94 (SEQ ID NO:223) reveals a signal sequence at about amino acid residues 1-16, a transmembrane domain at about residues 399-418, N-glycosylation sites at about amino acid residues 189-193 and 381-385, a glycosaminoglycan attachment site at about amino acid residues 289-293, cAMP- and cGMP-dependent protein kinase phosphorylation sites at about amino acid residues 98-102 and 434-438, N-myristolation sites about amino acid residues 30-36, 35-41, 58-64, 59-65, 121-127, 151-157, 185-191, 209-215, 267-273, 350-356, 374-380, 453-459, 463-469 and 477-483 and an aspartic acid and asparagine hydroxylation site at about amino acid residues 262-274.

AV. Isolation of cDNA Encoding Human PRO344 (UNQ303)

Use of the ECD homology procedure described above in a human fetal kidney library in combination with an *in vitro* cloning procedure using the probe oligonucleotide and one of the primer pairs below resulted in the identification of the full length DNA sequence DNA40592 (Fig. 95, SEQ ID NO:240) and the derived PRO344 native sequence protein UNQ303 (Fig. 96, SEQ ID NO:241).

The forward and reverse PCR primers and the hybridization probe used were the following:

forward PCR primer (34398.f1): (SEQ ID NO:242)
5'-TACAGGCCCAGTCAGGACCAGGGG-3'
forward PCR primer (34398.f2): (SEQ ID NO:243)
5'-AGCCAGCCTCGCTCTCGG-3'
forward PCR primer (34398.f3): (SEQ ID NO:244)
5'-GTCTGCGATCAGGTCTGG-3'
reverse PCR primer (34398.r1): (SEQ ID NO:245)
5'-GAAAGAGGCAATGGATTCGC-3'
reverse PCR primer (34398.r2): (SEQ ID NO:246)
5'-GACTTACACTTGCCAGCACAGCAC-3'
hybridization probe (34398.p1): (SEQ ID NO:247)
5'-GGAGCACCACCAACTGGAGGGTCCGGAGTAGCGAGCGCCCCGAAG-3'

Clone DNA40592 (SEQ ID NO:240) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 227-229 and ending at the stop codon (TAG) at nucleotide positions 956-958 (Figure 95). The predicted PRO344 polypeptide precursor (*i.e.*, UNQ303, SEQ ID NO:241) is 243 amino acids long (Figure 96), has a calculated molecular weight of 25,298 daltons and a pI of 6.44.

Analysis of the UNQ303 polypeptide of Figure 96 (SEQ ID NO:241) reveals a signal peptide at about amino acid residue 1-15, N-myristolation sites at about amino acid residues 11-17, 68-74, and 216-222 and a cell attachment site at about amino acid residues 77-80. A cDNA clone containing DNA40592 (SEQ ID NO:240) has been deposited with ATCC on November 21, 1997 and is assigned ATCC deposit no. 209492.

AX. Isolation of cDNA clones Encoding Human PRO333 (UNQ294)

Use of the ECD homology procedure in combination with an *in vivo* cloning procedure resulted in the identification of the partial length sequence DNA41374 (SEQ ID NO:248, Figure 97).

Clone DNA41374 (SEQ ID NO:248) contains an incomplete open reading frame with an apparent translation termination site (*i.e.*, stop codon, TGA) at nucleotide residues 1185-1187, as indicated in bolded underline. The predicted partial length PRO333 polypeptide (*i.e.*, UNQ294, SEQ ID NO:249) is 394 amino acids long, a calculate molecular weight of 43,725 daltons and a pI of 8.36.

Analysis of the UNQ294 (SEQ ID NO:249) polypeptide of Figure 98 reveals a signal sequence at about amino acid residues 1-14, a transmembrane domain at about residues 359-376, N-myristoylation sites at about amino acid residues 166-172, 206-212, 217-223, 246-252, 308-314, 312-318, 361-367 and an immunoglobulin and major histocompatibility complex proteins signature at amino acid residues 315-323. A cDNA clone containing DNA41374 has been deposited with the ATCC on _____ and as assigned ATCC deposit number _____.

AY. Isolation of cDNA clones Encoding Human PRO381 (UNQ322)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the identification of the full length DNA sequence DNA44194 (Fig. 99, SEQ ID NO:250) and the derived PRO381 native sequence protein UNQ322 (Fig. 100, SEQ ID NO:251).

The forward and reverse PCR primers and the hybridization probe used were the following:

Forward PCR primer (39651.f1): (SEQ ID NO:252)
5'-CTTTCCTTGCTTCAGCAACATGAGGC-3'
Reverse PCR primer (39651.r1): (SEQ ID NO:253)
5'-GCCCAGAGCAGGAGGAATGATGAGC-3'
hybridization probe (39651.p1): (SEQ ID NO:254)
5'-GTGGAACGCGGTCTTGACTCTGTTCGTCACTTCTTTGATTGGGGCTTTG-3'

Clone DNA44194 (SEQ ID NO:250) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 174-176 and ending at the stop codon (TAG) at nucleotide positions 807-809 (Fig. 99), as indicated by bolded underline. The predicted PRO381 polypeptide precursor (*i.e.*, UNQ322, Fig. 100, SEQ ID NO:251) is 211 amino acids long, has a calculated molecular weight of 24,172 daltons and has a pI of 5.99. The UNQ322 (SEQ ID NO:251) protein shown in Figure 100 has the following features: a signal peptide from about amino acid residues 1 to about 20, a potential N-glycosylation site at about amino acid residue 156, potential casein kinase phosphorylation sites from about amino acid residues 143 to about 146, about residues 156 to about 159, about residues 178 to about 181, about residues 200 to about 203, an endoplasmic reticulum targeting sequence from about amino acid residues 78 to about 114 and from about residues 118 to about 131, EF-hand calcium binding domain from about amino acid residues

140 to about 159, and an S-100/ICaBP type calcium binding domain from about amino acid residues 183 to about 203. A cDNA clone containing DNA44194 (SEQ ID NO:250) has been deposited with the ATCC on April 28. 1998 and is assigned deposit number 209808.

AZ. Isolation of cDNA clones Encoding Murine PRO720 (UNQ388)

The preparation of DNA53517 (SEQ ID NO:255) is described above under "X. Isolation of cDNA clones Encoding Human PRO770 (UNQ408)." Clone DNA53517 (SEQ ID NO:255) contains a single open reading frame with an apparent translation initiation site at nucleotide residues 36-38 and ending at the stop codon (TAA) at 369-371 (Figure 101), as indicated by bolded underline. The predicted PRO720 polypeptide precursor (*i.e.*, UNQ388, SEQ ID NO:256) is 111 amino acids long (Figure 102), has a calculated molecular weight of 11,936 daltons and a pI of 5.21.

Analysis of the UNQ388 (SEQ ID NO:256) polypeptide of Figure 102 reveals a signal sequence at about amino acid residues 1-23, N-myristolation sites at about amino acids residues 70-76 and 75-81 and, prokaryotic membrane lipoprotein lipid attachment sites at 66-77 and 68-79. A cDNA clone containing DNA53517 (SEQ ID NO:255) has been deposited with the ATCC on April 23. 1998 and is assigned deposit number 209802.

BA. Isolation of cDNA clones Encoding Human PRO866 (UNQ435)

Use of the ECD homology procedure described above in a human fetal kidney library resulted in the identification of the full length DNA sequence DNA53971 (Fig. 103, SEQ ID NO:257) and the derived PRO866 native sequence protein UNQ435 (Fig. 104, SEQ ID NO:258).

The forward and reverse PCR primers and the hybridization probe used were the following:

Forward PCR primer (44708.f1): (SEQ ID NO:259)
5'-CAGCACTGCCAGGGGAAGAGGG-3'

Forward PCR primer (44708.f2): (SEQ ID NO:260)
5'-CAGGACTCGCTACGTCCG-3'

Forward PCR primer (44708.f3): (SEQ ID NO:261)
5'-CAGCCCCTTCTCCTCCTTTCTCCC-3'

Reverse PCR primer (44708.r1): (SEQ ID NO:262)
5'-GCAGTTATCAGGGACGCACTCAGCC-3'

Reverse PCR primer (44706.r2): (SEQ ID NO:263)
5'-CCAGCGAGAGGCAGATAG-3'

Reverse PCR primer (44706.r3): (SEQ ID NO:264)
5'-CGGTCACCGTGTCCTGCGGGATG-3'

hybridization probe (44708.p1): (SEQ ID NO:265)
5'-CAGCCCCTTCTCCTCCTTTCTCCCACGTCCTATCTGCCTCTC-3'

The clone DNA53971 (SEQ ID NO:257) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 275-277 and ending at the stop codon (TAA) at nucleotide positions 1268-1270 (Figure 103), as indicated by bolded underline. The predicted native sequence PRO866 polypeptide precursor (*i.e.*, UNQ435, SEQ ID NO:258) is 331 amino acids (Figure 104), has a calculated

molecular weight of 35,844 daltons and a pI of 5.45. The UNQ435 (SEQ ID NO:258) protein shown in Figure 104 has an estimated molecular weight of about 35,844 daltons and a pI of about 5.45. Further analysis reveals a signal peptide from about amino acid residue 1 to about residue 26, glycosaminoglycan attachment sites at about amino acid residues 131-135, cAMP- and cGMP-dependent protein kinase phosphorylation sites at about amino acid residues 144-148 and N-myristoylation sites at amino acid residues 26-32, 74-80, 132-138, 134-140, 190-196, 287-293 and 290-296. A cDNA clone containing DNA53971 (SEQ ID NO:257) has been deposited with the ATCC on April 6, 1998 and is assigned deposit no. 209750.

BB. Isolation of cDNA clones Encoding Human PRO840 (UNQ433)

The use of a yeast screen procedure on tissue isolated from a human thyroid library resulted in an EST sequence which served as the template for the creation of PCR oligonucleotides which ultimately resulted in the isolation of DNA53987 (SEQ ID NO:266, Figure 105) and the derived PRO840 native sequence protein UNQ433 (SEQ ID NO:267, Figure 106).

A nucleotide sequence encoding a polypeptide of substantial homology with UNQ433 (SEQ ID NO:267) of Figure 106 is also available from GenBank as accession number HEEPSSARC_1.

DNA53987 (SEQ ID NO:266) as shown in Figure 105 contains an open reading frame with a translation initiation site at about nucleotide residues 18-20 and ending at the stop codon (TGA) at nucleotide residues 1329-1331, as indicated by bolded underline. The second methionine codon at nucleotide residues 90-92 could possibly also be the actual translation initiation site - alternatively, this codes for an internal methionine. The predicted PRO840 polypeptide (*i.e.*, the longer translation) has been termed UNQ433 (SEQ ID NO:267) and is 437 amino acids long (Figure 106), has a calculated molecular weight of 49,851 daltons and a pI of 6.47.

A cDNA clone containing DNA53987 (SEQ ID NO:266) has been deposited with the ATCC on May 12, 1998 under ATCC deposit number 209858.

Analysis of the UNQ433 polypeptide of Figure 106 (SEQ ID NO:267) reveals a signal sequence at about amino acid residues 1-46, a transmembrane domain at about amino acid residues 319-338, an N-glycosylation site at about residues 200-204, a cAMP and cGMP-dependent protein kinase phosphorylation sites at amino acid residues 23-27, tyrosine kinase phosphorylation sites at amino acid residues 43-52 and N-myristolylation sites at residues 17-23, 112-118, 116-122 and 185-191.

BC. Isolation of cDNA clones Encoding Human PRO982 (UNQ483)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence no. 43715. This sequence was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Merck EST No. AA024389. The full-length clone corresponding to this EST resulted in the identification of the full-length sequence DNA57700 (Fig. 107, SEQ ID NO:268) and the derived PRO982 native sequence protein UNQ483 (Fig. 108, SEQ ID NO:269).

The DNA57700 sequence of Figure 107 (SEQ ID NO:268) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 26-28 and ending at the stop codon (TAA) found at nucleotide positions 401-403, as indicated by bolded underline. The prediced PRO982 polypeptide precursor

(*i.e.*, UNQ982, SEQ ID NO:191) is 124 amino acids in length, has a calculated molecular weight of approximately 14,198 daltons and an estimated pI of approximately 9.01 (Fig. 108). Further analysis of the UNQ483 (SEQ ID NO:269) polypeptide of Figure 108 reveals a signal peptide from about amino acid residues 1 to about 21 and potential anaphylatoxin domain from about amino acid residue 1 to about residue 59. A cDNA clone containing DNA57700 (SEQ ID NO:268) was deposited with the ATCC on January 12, 1999 and is assigned ATCC deposit No. 203583.

BD. Isolation of cDNA clones Encoding Human PRO836 (UNQ545)

Use of the signal algorithm procedure described above resulted in the identification of EST clusters which were then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST 2610075, an EST derived from colon tumor tissue. The full-length clone corresponding to this EST resulted in the identification of the full-length sequence DNA59620 (Fig. 109, SEQ ID NO:270) and the derived PRO836 native sequence protein UNQ545 (Fig. 110, SEQ ID NO:271).

The nucleotide sequence DNA59620 (SEQ ID NO:270) shown in Figure 109 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 65-67 and ending at the stop codon (TGA) at nucleotide positions 1448-1450 (Fig. 109), as indicated by bolded underline. The predicted PRO836 polypeptide precursor (*i.e.*, UNQ545, Fig. 110, SEQ ID NO:271) is 461 amino acids in length. UNQ545 (SEQ ID NO:271) shown in Figure 110 has an estimated molecular weight of about 52,085 daltons and a pI of about 5.36. Further analysis reveals a signal peptide at about amino acid residues 1 to about 29, N-glycosylation sites at about amino acid residues 193 and 236 and N-myristoylation sites at about residues 15, 19, 234, 251, 402 and 451, a domain conserved in the YJL126w/YLR351c/yhcX family of proteins at about amino acid residues 364 to about 372, and a region having sequence identity with SLS1 protein at about amino acid residues 68 to about 340.

A cDNA clone containing DNA59620 (SEQ ID NO:270) has been deposited with the ATCC on 16 June 1998 and is assigned deposit number 209989.

BE. Isolation of cDNA clones Encoding Human PRO1159 (UNQ589)

Use of the signal algorithm procedure described above resulted in the identification of EST cluster sequence 77245, which was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST no. 376776. Analysis of the full-length clone corresponding to this EST resulted in the identification of the full-length sequence DNA60627 (Fig. 111, SEQ ID NO:272) and the derived PRO1159 native sequence protein UNQ589 (Fig. 112, SEQ ID NO:273).

Clone DNA60627 (SEQ ID NO:272) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 92-94 and ending at the stop codon (TAG) at nucleotide positions 362-364 (Figure 111), as indicated by bolded underline. The predicted PRO1159 polypeptide precursor (*i.e.*, UNQ589, SEQ ID NO:273) is 90 amino acids long (Figure 112). The UNQ589 (SEQ ID NO:273) protein shown in Figure 112 has an estimated molecular weight of about 9,840 daltons and a pI of about 10.13.

Analysis of the UNQ589 (SEQ ID NO:273) sequence shown in Figure 112 evidences the presence of the following: a signal peptide from about amino acid residue 1 to about residue 15 and a potential N-glycosylation site at about amino acid residue 38. Clone DNA60627 (SEQ ID NO:272) has been deposited with ATCC on August 4, 1998 and is assigned ATCC deposit no. 203092.

BF. Isolation of cDNA clones Encoding Human PRO1358 (UNQ707)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence, which was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST 088718, a fragment derived from a liver tissue library. Analysis of the full-length clone corresponding to the EST resulted in the identification of the full-length sequence DNA64890 (Fig. 113, SEQ ID NO:274) and the derived PRO1358 native sequence protein UNQ707 (Fig. 114, SEQ ID NO:275).

The DNA64890 (SEQ ID NO:274) clone shown in Figure 113 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 86 through 88 and ending at the stop codon (TAA) found at nucleotide positions 1418 through 1420 (Figure 113), as indicated by bolded underline. The predicted PRO1358 polypeptide precursor (*i.e.*, UNQ707, SEQ ID NO:275) is 444 amino acids long, and a signal peptide is at about amino acid residues 1-18. UNQ707 (SEQ ID NO:275) has a calculated molecular weight of approximately 50719 daltons and an estimated pI of approximately 8.82. A cDNA clone containing DNA64890 (SEQ ID NO:274), designated as DNA64890-1612, was deposited with the ATCC on August 18, 1998 and is assigned ATCC deposit no. 203131.

BG. Isolation of cDNA clones Encoding Human PRO1325 (UNQ685)

Use of the signal algorithm procedure described above resulted in the identification of the EST cluster sequence no. 139524, which was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of Incyte EST 3744079. Analysis of the full-length clone corresponding to the EST resulted in the identification of the full-length sequence DNA66659 (Fig. 115, SEQ ID NO:276) and the derived PRO1325 native sequence protein UNQ685 (Fig. 116, SEQ ID NO:277).

Clone DNA66659 (Fig. 115, SEQ ID NO:276) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 51-53 and ending at the stop codon (TAG) at nucleotide positions 2547-2549, as indicated by bolded underline. The predicted PRO1325 polypeptide precursor (*i.e.*, UNQ685, SEQ ID NO:227) is 832 amino acids long. The UNQ685 (SEQ ID NO:227) protein shown in Figure 116 has an estimated molecular weight of about 94,454 daltons and a pI of about 6.94. Further analysis of UNQ685 (SEQ IDNO:227) reveals: a signal peptide from about amino acid 1 to about amino acid 18, transmembrane domains from about amino acid 292 to about amino acid 317, from about amino acid 451 to about amino acid 470, from about amino acid 501 to about amino acid 520, from about amino acid 607 to about amino acid 627 from about amino acid 751 to about amino acid 770, a leucine zipper pattern sequence from about amino acid 497 to about amino acid 518 and potential N-glycosylation sites from about amino acid 27 to about amino acid 30, from about amino acid 54 to about amino acid 57, from about amino acid 60 to about amino acid 63, from about amino acid position 123 to about amino acid position 126, from about amino

acid position 141 to about amino acid position 144, from about amino acid position 165 to about amino acid position 168, from about amino acid position 364 to about amino acid position 367, from about amino acid position 476 to about amino acid position 479, from about amino acid position 496 to about amino acid position 499, from about amino acid position 572 to about amino acid position 575, from about amino acid position 603 to about amino acid position 606 and from about amino acid position 699 to about amino acid position 702. A cDNA clone containing DNA66659 (SEQ ID NO:276)) has been deposited with ATCC on September 22, 1998 and is assigned ATCC deposit no. 203269.

BH. Isolation of cDNA clones Encoding Human PRO1338 (UNQ693)

The use of yeast screens resulted in EST sequences which were then compared to various public and private EST databases in a manner similar to that described above under ECD homology resulted in the identification of Incyte EST2615184, an EST derived from cholecystitis gall bladder tissue. Analysis of the corresponding full-length sequence ultimately resulted in the isolation of DNA66667 (SEQ ID NO:278, Figure 117) and the derived PRO1338 native sequence protein UNQ693 (SEQ ID NO:279, Figure 118).

DNA66667 (SEQ ID NO:278) as shown in Figure 117 contains a single open reading frame with a translation initiation site at about nucleotide residues 115-117 and ending at the stop codon (TAA) at nucleotide positions 2263-2265, as indicated by bolded underline. The predicted PRO1338 polypeptide precursor (*i.e.*, UNQ693, SEQ ID NO:118) is 716 amino acids in length (Figure 118), has a calculated molecular weight of 80,716 daltons and a pI of 6.06.

Analysis of the UNQ693 polypeptide (SEQ ID NO:278) of Figure 118 reveals a signal sequence at about amino acid residues 1 to 25, a transmembrane domain at about amino acid residues 629-648, N-glycosylation sites at about amino acid residues 69-73, 96-100, 106-110, 117-121, 385-389, 517-521, 582-586 and 611-615, a tyrosine kinase phosphorylation site at about residues 573-582 and N-myristoylation sites at about amino acid residues 16-22, 224-230, 464-470, 637-643 and 698-704.

A cDNA containing DNA66667 (SEQ ID NO:278) has been deposited with the ATCC under the designation DNA66667-1596 on September 22, 1998 and has been assigned ATCC deposit number 203267.

BI. Isolation of cDNA clones Encoding Human PRO1434 (UNQ739)

Use of ECD homology procedure described above in a human retina tissue library resulted in the identification of the full-length DNA sequence DNA68818 (Fig. 119, SEQ ID NO:280) and the derived PRO1434 native sequence protein UNQ739 (Fig. 120, SEQ ID NO:281).

The PCR primers (forward and reverse) and hybridization probe synthesized in this procedure were the following:

forward PCR primer:

5'-GAGGTGTCGCTGTGAAGCCAACGG-3' (SEQ ID NO:282)

reverse PCR primer:

5'-CGCTCGATTCTCCATGTGCCTTCC-3' (SEQ ID NO:283)

hybridization probe: (SEQ ID NO:284)

5'-GACGGAGTGTGTGGACCCTGTGTACGAGCCTGATCAGTGCTGTCC-3'

Clone DNA68818 (SEQ ID NO:280) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 581-583 and ending at the stop codon (TAG) at nucleotide positions 1556-1558 (Figure 119), as indicated by bolded underline. The predicted PRO1434 polypeptide precursor (*i.e.*, UNQ739, SEQ ID NO:281) is 325 amino acids long (Figure 120). The UNQ739 (SEQ ID NO:281) protein shown in Figure 120 has an estimated molecular weight of about 35,296 daltons and a pI of about 5.37. Further analysis reveals a signal sequence at about amino acid residues 1-27, a glycosaminoglycan attachment site at about amino acid residues 80-84, M-myristoylation sites at about amino acid residues 10-16, 102-108, 103-109, a cell attachment sequence at about amino acid residues 114-117 and an EGF-like domain cysteine pattern signature at about amino acid residues 176-188.

A clone containing DNA68818 (SEQ ID NO:280) has been deposited with ATCC under the designation DNA68818-2536 on February 9, 1999 and is assigned ATCC deposit no. 203657.

BJ. Isolation of cDNA clones Encoding Human PRO4333 (UNQ1888)

An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched in a manner similar to that described above under the ECD homology procedure described above and an EST was identified which showed homology to lymphotoxin-beta receptor.

The EST served as the template to create oligonucleotide primers and probes to screen a human fetal kidney library in a manner similar to that described above under the ECD homology procedure.

The oligonucleotides created for the above procedure were the following:

forward PCR primer: (SEQ ID NO:287)
5'-GCAAGAATTCAGGGATCGGTCTGG-3'
probe: (SEQ ID NO:288)
5'-CTGTGTTCCCTGCAACCAGTGTGGGCCAGGCATGG AGTTGTCTAAGG-3'
reverse: (SEQ ID NO:289)
5'-AGATGGCATCACTG GTGGCTGAAC-3'
forward: (SEQ ID NO:290)
5'-CAGAAGGCAAATTGTTCAGCCACCAG-3'
reverse: (SEQ ID NO:291)
5'-ACAGTTTCCAGACCGATCCCTGAATTC-3'

The result was the isolation of the full-length DNA sequence DNA84210 (SEQ ID NO:285, Figure 121). The DNA84210 (SEQ ID NO:285) clone depicted in Figure 121 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 185-187, and a stop codon (TAA) at nucleotide positions 1436-1438, as indicated by bolded underline. The predicted PRO4333 polypeptide precursor (*i.e.*, UNQ1888, SEQ ID NO:286) is 417 amino acids long. The UNQ1888 protein (SEQ ID NO:286) shown in Figure 121 has an estimated molecular weight of about 45305 daltons and a pI of about 5.12.

Analysis of the UNQ1888 polypeptide (SEQ ID NO:286) of Figure 121 reveals a signal peptide at about amino acid residues 1-25, a transmembrane domain at about residues 169-192, N-glycosylation sites about residues 105-109, 214-218, 319-323, 350-354, 368-372, 379-383, cAMP- and cGMP-dependent protein kinase phosphorylation sites at about residues 200-204 and 238-242, a tyrosine kinase phosphorylation site at

about residues 207-214, an N-myristoylation site at about residues 55-61, 215-218 and 270-276, a prokaryotic membrane lipoprotein lipid attachment site at about residues 259-270 and a TNFR/NGFR family cysteine-rich region at about residues 89-96.

A cDNA clone containing DNA84210 (SEQ ID NO:285), designated as DNA84210-2576, has been deposited with ATCC on March 2, 1999 and is assigned ATCC deposit no. 203818.

BK. Isolation of cDNA clones Encoding Human PRO4302 (UNQ1866)

Use of the amylase screen procedure described above on tissue isolated from human tissue resulted in an EST sequence which was then compared against various EST databases to create a consensus sequence by a methodology as described above under the amylase yeast screen procedure and/or the ECD homology procedure. Further analysis of this consensus sequence resulted in the identification of Incyte EST no. 2408081H1. Analysis of the full-length clones corresponding to EST no. 2408081H1 resulted in the isolation of the full length native sequence clones DNA92218 (SEQ ID NO:292) and the derived PRO4302 full-length native sequence protein UNQ1866 (SEQ ID NO:293).

The full length clone DNA92218 (SEQ ID NO:292) shown in Figure 123 has a single open reading frame with an apparent translational initiation site at nucleotide positions 174-176 and a stop signal (TAG) at nucleotide positions 768-770, as indicated by bolded underline. The predicted PRO4302 polypeptide precursor (*i.e.*, UNQ1866, SEQ ID NO:293) is 198 amino acids long, has a calculated molecular weight of approximately 22,285 daltons and an estimated pI of approximately 9.35. Analysis of UNQ1866 (Fig. 124, SEQ ID NO:293) reveals a signal peptide from about amino acid residue 1 to about residue 23, a transmembrane domain from about amino acid residue 111 to about residue 130, a cAMP and cGMP-dependent protein kinase phosphorylation sites at residues 26-30, casein kinase II phosphorylation sites at residues 44-47 and 58-61, a tyrosine kinase phosphorylation site at residues 36-43 and N-myristoylation sites at residues 124-130, 144-150 and 189-195.

A cDNA clone containing DNA92218 (SEQ ID NO:292), designated DNA92218-2554, was deposited with the ATCC on March 9, 1999 and has been assigned deposit number 203834.

BL. Isolation of cDNA clones Encoding Human PRO4430 (UNQ1947)

Use of the signal algorithm procedure described above resulted in the identification of an EST cluster sequence, which was then compared to a variety of various EST databases as described under the signal algorithm procedure above, and further resulted in the identification of a consensus sequence. Further analysis of the consensus sequence resulted in the identification of the full-length sequence DNA96878 (Fig. 125, SEQ ID NO:294) and the derived PRO4430 native sequence protein UNQ1947 (Fig. 126, SEQ ID NO:295).

The native sequence DNA sequence DNA96878 (SEQ ID NO:294) shown in Figure 125 contains a single open reading frame with an apparent translation initiation site at nucleotide positions 56-58 and ending at the stop codon (TGA) found at nucleotide positions 431-433, as indicated by bolded underline. The predicted PRO4430 polypeptide precursor (UNQ1947, Fig. 126, SEQ ID NO:295) is 125 amino acids long. The UNQ4430 protein (SEQ ID NO:295) of Figure 126 has a calculated molecular weight of approximately 13821 daltons and an estimated pI of approximately 8.6. Further analysis reveals the presence of a signal sequence at about amino acid residues 1 to about 18, N-glycosylation sites at about residues 77-80 and again at

about residues 88-91, a casein kinase II phosphorylation site at about residues 67-70, an N-myristoylation site at about residues 84-89 and a Lys-6/u-PAR domain at about residues 85-98.

A clone containing DNA96878 (SEQ ID NO:294), designated DNA96878-2626, was deposited with the ATCC on May 4, 1999 and is assigned ATCC deposit no. 23-PTA.

BM. Isolation of cDNA clones Encoding Human PRO5727 (UNQ2448)

Various known TNF-receptors were used to screen public and private EST databases (*e.g.*, see ECD homology procedure, above) resulting in the identification Incyte clone 509151H1H. This EST sequence, which was derived from uterine tumor tissue, then served as a template for the construction of the cloning oligos indicated below which were then used to identify by PCR a human thymus cDNA library that contained the sequence of interest. These oligonucleotides were:

Forward primer (509-1):

5'-GAGGGGGCTGGGTGAGATGTG-3' (SEQ ID NO:298)

Reverse primer (509-4AS):

5'-TGCTTTTGTACCTGCGAGGAGG-3' (SEQ ID NO:299)

To isolate the DNA sequence encoding the full-length DNA98853 polypeptide, an inverse long distance PCR procedure was carried out (Figure 129). The PCR primers generally ranged from 20 to 30 nucleotides. For inverse long distance PCR, primer pairs were designed in such a way that the 5' to 3' direction of each primer pointed away from each other.

A pair of inverse long distance PCR primers for cloning DNA98853 were synthesized:

Primer 1 (left primer) (509-P5):

5'-pCATGGTGGGAAGGCCGGTAACG-3' (SEQ ID NO:300)

Primer 2 (right primer) (509-P6):

5'-pGATTGCCAAGAAAATGAGTACTGGGACC-3' (SEQ ID NO:301)

In the inverse long distance PCR reaction, the template is the plasmid cDNA library. As a result, the PCR products contain the entire vector sequence in the middle with insert sequences of interest at both ends. After the PCR reaction, the PCR mixture was treated with Dpn I which digests only the template plasmids, followed by agarose gel purification of PCR products of larger than the size of the library cloning vector. Since the primers used in the inverse long distance PCR were also 5'-phosphorylated, the purified products were then self-ligated and transformed into *E.coli* competent cells. Colonies were screened by PCR using 5' vector primer and proper gene specific primer to identify clones with larger 5' sequence. Plasmids prepared from positive clones were sequenced. If necessary, the process could be repeated to obtain more 5' sequences based on new sequence obtained from the previous round.

The purpose of inverse long distance PCR is to obtain the complete sequence of the gene of interest. The clone containing the full length coding region was then obtained by conventional PCR.

The primer pair used to clone the full length coding region of DNA98853 (SEQ ID NO:296) were the following:

Forward primer (Cla-MD-509):

5'-GGAGGATCGATACCATGGATTGCCAAGAAAATGAG-3' (SEQ ID NO:302)

Reverse primer (509.TAA.not):

5'-GGAGGAGCGGCCGCTTAAGGGCTGGGAACTTCAAAGGGCAC-3' (SEQ ID NO:303)

For cloning purposes, a Cla I site and a Not I site were included in the forward primer and reverse primer respectively.

To ensure the accuracy of the PCR products, independent PCR reactions were performed and several cloned products were sequenced.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for DNA98853 (SEQ ID NO:296, Figure 127) and the derived PRO5727 native sequence protein UNQ2448 (SEQ ID NO:297, Figure 128).

Clone DNA98853 (SEQ ID NO:296) contains a single open reading frame with an apparent translation initiation site at nucleotide positions 1-3 and ending at the stop codon (TAA) at nucleotide positions 901-903 (Figure 127), as indicated by bolded underline. The predicted PRO5727 polypeptide precursor (*i.e.*, UNQ2448, SEQ ID NO:297) is 299 amino acids long (Figure 128), has a calculated molecular weight of 32,929 daltons and a pI of 4.95. The UNQ2448 polypeptide (SEQ ID NO:297) shown in Figure 128 has an estimated molecular weight of about 3.3 kilodaltons and a pI of about 4.72. A potential N-glycosylation site exists between amino acids 74 and 77 of the amino acid sequence shown in Figure 128. A potential N-myristoylation site exists between amino acids 24 and 29 of the amino acid sequence shown in Figure 128. Potential casein kinase II phosphorylation sites exist between amino acids 123-126, 185-188, 200-203, 252-255, 257-260, 271-274, and 283-286 of the amino acid sequence shown in Figure 128. A potential transmembrane domain exists between amino acids 137 to 158 of the sequence shown in Figure 128. It is presently believed that the polypeptide does not include a signal sequence.

A cDNA clone containing DNA98853 (SEQ ID NO:296, designated DNA98853-1739, has been deposited with ATCC on April 6, 1999 and is assigned ATTC Deposit No. April 6, 1999.

EXAMPLE 2

Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no.24)

This example shows that the polypeptides of the invention are active as a stimulator of the proliferation of stimulated T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may take the form of antagonists of the polypeptide of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO_2)and then washed and resuspended to 3 x 10^6 cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate).

The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads). The assay is prepared by plating in triplicate wells a mixture of: 100µl of test sample diluted to 1% or to 0.1%; 50 µl of irradiated stimulator cells and 50 µl of responder PBMC cells. 100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO_2 for 4 days. On day 5 and each well is pulsed with tritiated thymidine (1.0 mC/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI;10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids. 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to 1x 10^7 cells/ml of assay media. The assay is then conducted as described above. The results of this assay for compounds of the invention are shown below. Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

Table 7

PRO	PRO Concentration	Percent Increase Over Control
PRO356	0.1%	133.8
PRO356	0.1%	208.9
PRO356	1.0%	251.6
PRO356	1.0%	332.1
PRO273	12.4 nM	112
PRO273	124 nM	192.7
PRO769	23.86 nM	76.3
PRO769	238.6 nM	226
PRO1184	16.88 nM	81.6
PRO1184	168.82 nM	194.4
PRO1346	3.34 nM	86.6
PRO1346	33.41 nM	188.5
PRO1246	0.07 nM	145
PRO1246	0.7 nM	180.9
PRO269	0.1%	122.4
PRO269	1%	194.1
PRO344	0.1%	148.6
PRO344	1%	259.9
PRO333	0.1%	187.8
PRO333	1%	220
PRO381	14.5 nM	87.3
PRO381	14.5 nM	135.4

PRO381	145 nM	248.1
PRO381	145 nM	290.8
PRO533	0.06 nM	163
PRO533	0.61 nM	382.9
PRO720	0.1 nM	198.4
PRO720	1.0 nM	293.5
PRO866	0.1 nM	131.8
PRO866	1.04 nM	223.2

EXAMPLE 3

Hairless Guinea pig Proinflammatory Assay (no. 32)

This assay is designed to determine whether the PRO polypeptides show the ability to induce vascular permeability. Polypeptides testing positive in this assay are expected to be useful for the therapeutic treatment of conditions which would benefit from enhanced vascular permeability including, for example, conditions which may benefit from enhanced local immune system cell infiltration.

Hairless guinea pigs weighing 350 grams or more were anesthetized with Ketamine (75-80 mg/kg) and 5 mg/kg Xylazine intramuscularly. Test samples containing the PRO polypeptide or a physiological buffer without the test polypeptide are injected into skin on the back of the test animals with 100 µl per injection site intradermally. There were approximately 16-24 injection sites per animal. One ml of Evans blue dye (1% in PBS) is then injected intracardially. Skin vascular permeability responses to the compounds (*i.e.*, blemishes at the injection sites of injection) are visually scored by measuring the diameter (in mm) of blue-colored leaks from the site of injection at 1, 6 and/or 24 hours post administration of the test materials. The mm diameter of blueness at the site of injection is observed and recorded as well as the severity of the vascular leakage for values scoring above 4 standard deviations over the same animal control. Blemishes of at least 5 mm in diameter are considered positive for the assay when testing purified proteins, being indicative of the ability to induce vascular leakage or permeability. A response greater than 7 mm diameter is considered positive for conditioned media samples. Human VEGF is used as a positive control, inducing a response of 4-8 mm diameter at 0.1 µg/100 µl., and 15-23 mm diam. at 1 µg/100 µl.

The tested polypeptide are diluted to 1% of the initial stock solution. UNQ 585 was diluted into 10 mM HEPES/140 mM NaCl/4% mannitol/1 mg/ml BSA pH 6.8, while UNQ334 was diluted into 140 mM NaCl, 10 mM Hepes, 4% Mannitol pH 7.4.

Table 8

UNQ polypeptide	Stock solution concentration	Time (hr)	dialation (mm)
PRO1155	20,384 nM	1	6
PRO1155	20,384 nM	6	6
PRO533	1024 nM	1	5.4
PRO533	1024 nM	6	7
PRO21	22,000 nM	1	2.0
PRO21	22,000 nM	6	14.0

EXAMPLE 4

Skin Vascular Permeability Assay (no.64)

This assay shows that certain PRO polypeptides stimulate an immune response and induce inflammation by inducing mononuclear cell, eosinophil and PMN infiltration at the site of injection of the animal. This skin vascular permeability assay is conducted as follows. Hairless guinea pigs weighing 350 grams or more are anesthetized with ketamine (75-80 mg/Kg) and 5 mg/Kg Xylazine intramuscularly (IM). A sample of purified PRO polypeptide or a conditioned media test sample is injected intradermally onto the backs of the test animals with 100 uL per injection site. It is possible to have about 10-30, preferably about 16-24, injection sites per animal. One mL of Evans blue dye (1% in physiologic buffered saline) is injected intracardially. Blemishes at the injection sites are then measured (mm diameter) at 1hr, 6 hrs and 24 hrs post injection. Animals were sacrificed at 6 hrs after injection. Each skin injection site is biopsied and fixed in paraformaldehyde. The skins are then prepared for histopathalogic evaluation. Each site is evaluated for inflammatory cell infiltration into the skin. Sites with visible inflammatory cell inflammation are scored as positive. Inflammatory cells may be neutrophilic, eosinophilic, monocytic or lymphocytic

At least a minimal perivascular infiltrate at the injection site is scored as positive, no infiltrate at the site of injection is scored as negative.

Table 9

UNQ	Time (hrs)	Infiltrate
PRO172	24	positive
PRO200	24	positive
PRO200	24	positive
PRO216	24	positive
PRO272	24	positive
PRO362	24	positive
PRO1007	24	positive
PRO1031	24	positive
PRO1283	24	positive
PRO1343	24	positive
PRO1358	6	positive
PRO1325	6	positive
PRO1434	24	positive
PRO4333	6	positive

EXAMPLE 5

Inhibitory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no. 67)

This example shows that one or more of the PRO polypeptides are active as inhibitors of the proliferation of stimulated T-lymphocytes. Compounds which inhibit proliferation of lymphocytes are useful therapeutically where suppression of an immune response is beneficial.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO_2) and then washed and resuspended to $3x10^6$ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:l of test sample diluted to 1% or to 0.1%,

50 :l of irradiated stimulator cells, and

50 :l of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO_2 for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mC/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to $1x10^7$ cells/ml of assay media. The assay is then conducted as described above.

Any decreases below control is considered to be a positive result for an inhibitory compound, with decreases of less than or equal to 80% being preferred. However, any value less than control indicates an inhibitory effect for the test protein.

Table 10

PRO	PRO Concentration	Percent Decrease Below Control
PRO204	0.1%	86
PRO204	1.0%	35
PRO212	0.59 nM	0
PRO212	5.9 nM	52.6
PRO212	0.87 nM	82.7
PRO212	8.7 nM	66
PRO212	1.9 nM	81.6
PRO212	19 nM	61.5
PRO212	0.46 nM	66.1
PRO212	4.6 nM	59.5
PRO212	2.1 nM	0

PRO212	2.1 nM	116.2
PRO212	21 nM	0
PRO212	21 nM	62.2
PRO216	0.13 nM	74.3
PRO216	1.3 nM	63.3
PRO226	0.12 nM	67.9
PRO226	1.2 nM	40.6
PRO235	0 nM	83.6
PRO235	0.02 nM	69.7
PRO240	5.3 nM	68.2
PRO240	53 nM	68.2
PRO240	35 nM	72.2
PRO240	350 nM	64
PRO245	19.1 nM	53
PRO245	191 nM	54
PRO245	0.93 nM	71.8
PRO245	0.93 nM	80.9
PRO245	9.3 nM	49.6
PRO245	9.3 nM	51.9
PRO273	31.46 nM	81
PRO273	314.56 nM	67
PRO332	0.35 nM	74.2
PRO332	3.5 nM	68
PRO332	0.35 nM	20.2
PRO332	3.5 nM	61.2
PRO361	1.5 nM	63.2
PRO361	15 nM	64.7
PRO363	8.6 nM	76.9
PRO363	86 nM	63.6
PRO363	8.6 nM	64.4
PRO363	86 nM	2.1
PRO364	0.31 nM	68.1
PRO364	3.1 nM	67.4
PRO364	1.7 nM	92.8
PRO364	17 nM	68.4
PRO364	1.7 nM	94.2
PRO364	17 nM	63.3
PRO526	0.12 nM	68.5
PRO526	1.2 nM	62.5
PRO531	0.2 nM	66.1

	PRO531	2 nM	54.3
	PRO531	0.2 nM	70.4
	PRO531	2 nM	68.4
	PRO701	0.74 nM	72.5
5	PRO701	0.74 nM	90.2
	PRO701	7.4 nM	64.8
	PRO701	7.4 nM	69
	PRO770	0.69 nM	65.8
	PRO770	6.9 nM	67.4
10	PRO788	12.96 nM	88.4
	PRO788	129.6 nM	57.7
	PRO788	2.9 nM	64.4
	PRO788	29 nM	67.4
	PRO865	0.27 nM	67.9
15	PRO865	2.7 nM	63.7
	PRO1083	7.1 nM	80.5
	PRO1083	71 nM	63.7
	PRO1083	7.1 nM	40.9
	PRO1083	71 nM	65
20	PRO1114	0.37 nM	44.9
	PRO1114	3.7 nM	42.4
	PRO1192	12.1 nM	31.6
	PRO1192	121 nM	32.6
	PRO1195	0.5 nM	67
25	PRO1195	5 nM	66.8
	PRO1250	0.05 nM	75.4
	PRO1250	0.5 nM	57.2
	PRO1250	0.05 nM	94.6
	PRO1250	0.5 nM	61.2
30	PRO1312	8.5 nM	52
	PRO1312	85 nM	49.3
	PRO1312	14.2 nM	73.1
	PRO1312	142 nM	62.9
	PRO1287	0.8 nM	79.1
35	PRO1387	8 nM	52.3
	PRO1410	4 nM	89
	PRO1410	40 nM	64.8
	PRO1418	6.4 nM	67.7
	PRO1418	6.4 nM	81.1
40	PRO1418	64 nM	56.3

PRO1418	64 nM	64.9
PRO1868	39.4 nM	65.8
PRO1868	394 nM	50
PRO1917	2.1 nM	70.7
PRO1917	2.1 nM	82.5
PRO1917	21 nM	60.7
PRO1917	21 nM	62.6
PRO205	0.7 nM	71.5
PRO205	7 nM	3.5
PRO840	24.4 nM	137.2
PRO982	244 nM	58.9
PRO836	2.5 nM	60.7
PRO836	25 nM	60.6
PRO1159	11.06 nM	80.4
PRO1159	110.55 nM	57.6
PRO1159	11.06 nM	81.9
PRO1159	110.55 nM	46.2
PRO1338	0.14 nM	80.7
PRO1338	1.4 nM	65.5
PRO4302	13.56 nM	115.8
PRO4302	135.57 nM	2.4
PRO4430	24.2 nM	55.9
PRO4430	242 nM	49.9
PRO5727	19.6 nM	69.2
PRO5727	196 nM	54.5

EXAMPLE 6

In situ Hybridization

In situ hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis and aid in chromosome mapping.

In situ hybridization was performed following an optimized version of the protocol by Lu and Gillett, *Cell Vision* 1: 169-176 (1994), using PCR-generated ^{33}P-labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinated in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A [^{33}P] UTP-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2 nuclear track emulsion and exposed for 4 weeks.

^{33}P-Riboprobe synthesis

6.0 µl (125 mCi) of ^{33}P-UTP (Amersham BF 1002, SA<2000 Ci/mmol) were speed vac dried. To each tube containing dried^{33}P-UTP, the following ingredients were added: 2.0 µl 5x transcription buffer; 1.0 µl DTT (100 mM); 2.0 µl NTP mix (2.5 mM : 10 µl; each of 10 mM GTP, CTP & ATP + 10 µl H_2O); 1.0 µl UTP (50 µM); 1.0 µl Rnasin; 1.0 µl DNA template (1µg); 1.0 µl H_2O.

The tubes were incubated at 37°C for one hour. 1.0 µL RQ1 DNase were added, followed by incubation at 37°C for 15 minutes. 90 µL TE (10 mM Tris pH 7.6/1mM EDTA pH 8.0) were added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a Microcon-50 ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, 100 µL TE were added. 1 µL of the final product was pipetted on DE81 paper and counted in 6 ml of Biofluor II.

The probe was run on a TBE/urea gel. 1-3 µL of the probe or 5 µL of RNA Mrk III were added to 3 µL of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, the sample loaded, and run at 180-250 volts for 45 minutes. The gel was wrapped in saran wrap and exposed to XAR film with an intensifying screen in -70°C freezer one hour to overnight.

^{33}P-Hybridization

Pretreatment of frozen sections The slides were removed from the freezer, placed on aluminum trays and thawed at room temperature for 5 minutes. The trays were placed in 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975 ml SQ H_2O). After deproteination in 0.5 µg/ml proteinase K for 10 minutes at 37°C (12.5µL of 10 mg/ml stock in 250 ml prewarmed RNase-free RNAse buffer), the sections were washed in 0.5 x SSC for 10 minutes at room temperature. The sections were dehydrated in 70%, 95%, 100% ethanol, 2 minutes each.

Pretreatment of paraffin-embedded sections The slides were deparaffinized, placed in SQ H_2O, and rinsed twice in 2 x SSC at room temperature, for 5 minutes each time. The sections were deproteinated in 20 µg/ml proteinase K (500 µL of 10 mg/ml in 250 ml RNase-free RNase buffer; 37C, 15 minutes) - human embryo, or 8 x proteinase K (100 µL in 250 ml Rnase buffer, 37°C, 30 minutes) - formalin tissues. Subsequent rinsing in 0.5 x SSC and dehydration were performed as described above.

Prehybridization The slides were laid out in plastic box lined with Box buffer (4 x SSC, 50% formamide) - saturated filter paper. The tissue was covered with 50 µL of hybridization buffer (3.75g Dextran Sulfate + 6 ml SQ H_2O), vortexed and heated in the microwave for 2 minutes with the cap loosened. After cooling on ice, 18.75 ml formamide, 3.75 ml 20 x SSC and 9 ml SQ H_2O were added, the tissue was vortexed well, and incubated at 42°C for 1-4 hours.

Hybridization 1.0 x 10^6 cp. probe and 1.0 µL RNA (50 mg/ml stock) per slide were heated at 95°C for 3 minutes. The slides were cooled on ice, and 48 µL hybridization buffer were added per slide. After vortexing, 50 µL ^{33}P mix were added to 50 µL prehybridization on slide. The slides were incubated overnight at 55C.

Washes Washing was done 2x10 minutes with 2xSSC, EDTA at room temperature (400 ml 20 x SSC + 16 ml 0.25M EDTA, V_f=4L), followed by RNaseA treatment at 37°C for 30 minutes (500 µL of 10 mg/ml in 250 ml Rnase buffer - 20 µg/ml), The slides were washed 2x10 minutes with 2 x SSC, EDTA at room temperature. The stringency wash conditions were as follows: 2 hours at 55°C, 0.1 x SSC, EDTA (20 ml 20 x SSC + 16 ml EDTA, V_f=4L).

Alternatively, multi-tissue blots containing poly A^+ RNA (2 µg per lane) from various human tissues were purchased from Clontech (Palo Alto, CA). DNA probes were labeled with [α-^{32}P]dCTP by random priming DNA labeling Beads (Pharmacia Biotech). Hybridization was performed with Expresshyb (Clontech) at 68°C for 1 hr. The blots were then washed with 2X SSC/0.05% SDS solution at room temperature for 40 min, followed by washes in 0.1X SSC/0.1%SDS solution at 55°C for 40 min with one change of fresh solution. The blots were exposed in a phosphorimager.

DNA 29101 (VEGFB9)

DNA29101 (SEQ ID NO:1) was examine in three separate *in situ* studies wherein the following probes were used:

VEGFB9-p1 (SEQ ID NO:194):

5'-GGATTCTAATACGACTCACTATAGGGCGGCGGAATCCAACCTGAGTAG-3'

VEGFB9-p2 (SEQ ID NO:195):

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GCG GCT ATC CTC CTG TGC TC-3'

IS97-029:

Expression observed in the developing lower fetal limb bones at the edge of the cartilagenous anlage (*i.e.*, around the outside edge); in developing tendons, in vascular smooth muscle and in cells embracing developing skeletal muscle myocytes and myotubes. Expression also observed in the following tissues: epiphyseal growth plate: lymph nodes - marginal sinus: thymus - subcapsular region of the thymic cortex, possibly representing either the subcapsular epithelial cells or the proliferating, double negative, thymocytes that are found in this region; tracheal smooth muscle; brain (cerebral cortex) - focal expression in cortical neurones; small intestine - smooth muscle; thyroid - thyroid epithelium; liver - ductal plates; stomach - mural smooth muscle; fetal skin - basal layer of squamous epithelium; placenta - interstitial cells in trophoblastic villi; spinal cord - no expression except in wall of arteries and veins. No expression was observed in the spleen and adrenals.

The above expression pattern suggests that DNA29101 may be involved in cell differentiation /proliferation.

IS97-037:

Expression in superovulated rat ovaries were negative in all sections with both antisense and sense probes. Either the message is not expressed in this model, or the human probe does not cross react with rat.

IS97-087:

High expression levers were observed at the following sites: chimp ovary - granulosa cells of maturing follicles, lower intensity signal observed over thecal cells; chimp parathyroid - high expression over chief cells; human fetal testis - moderate expression over stromal cells surrounding developing tubules: human fetal lung - high expression over chondrocytes in developing bronchial tree, and low level expression over branching bronchial epithelium.

Fetal tissues examined (E12-E16 weeks) include: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb. Adult tissues examined include liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung, skin, cerebral cortex (rm), hippocampus(rm), cerebellum(rm), penis, eye, bladder, stomach, gastric carcinoma, colon, colonic carcinoma and chondrosarcoma. Also examined were acetaminophen induced liver injury and hepatic cirrhosis

DNA30871:

IS97-044: In fetal tissues, strong signals were observed over neurones in fetal cerebral cortex, spinal cord, spinal ganglia as well as enteric neurones in the wall of the fetal stomach. Signal also observed over cells around the root of the aorta (possibly the conducting system), adrenal medulla, mesenchymal cells in neurovascular bundle, renal parenchyma and cells lying between skeletal muscle myocytes. All other fetal tissues negative.

No expression was observed in adult tissue. Fetal tissues (12-16 weeks) examined include: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb. Adult tissues examined include: liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung and skin.

The probes used in the above analysis were the following:

DNA30871-p1 (SEQ ID NO:196):

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CTC CCG TCT CCT CCT GTC CTC-3'

DNA30871-p2 (SEQ ID NO:197):

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA CCT CGG CAT CTT CGT CAC ATT-3'

DNA30942:

DNA30942 (SEQ ID NO:13) was examined in four separate *in situ* studies (including two in the diseased tissue study of Example 7 using the following probes:

DNA30942-p1 (SEQ ID NO:198)

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC TCG CTG CTG TGC CTG GTG TTG-3'

DNA30942-p2: (SEQ ID NO:199)

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA CCG CTG CAG CCT CTT GAT GGA-3'

IS97-043:

No expression was observed in fetal tissues. The fetal tissues examined included: placenta, umbilical cord, brain, spinal cord, eye, optic nerve, trachea, lung, heart, thymus, liver, spleen, esophagus, small intestine, pancreas, adrenal, thyroid, body wall and lower limb.

No expression was observed in adult tissues. The adult tissues examined included liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung and skin.

DNA33087 *(IS97-051)*:

In fetal tissue, expression of DNA33087 (SEQ ID NO:18) was observed in osteoblasts at all sites of enchondral and periosteal new bone formation, the developing pulmonary arterial and aortic trunks. The fetal tissues examined included: placenta, umbilical cord, brain, spinal cord, eye, optic nerve, trachea, lung, heart, thymus, liver, spleen, esophagus, small intestine, pancreas, adrenal, thyroid, body wall and lower limb.

No expression was observed in the adult tissues examined including: Liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung and skin.

The probable role in control of bone matrix deposition and or osteoblast growth.

All adult tissues in the multiblock were positive for beta-actin.

The probes used in this procedure were the following:

DNA33087-p1 (SEQ ID NO:200):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CCC GAG TGT TTT CCA AGA-3'
DNA33087-p2 (SEQ IDNO:201):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA CAA GTT TAC TAG CCC ATC CAT-3'
DNA33087-p3 (SEQ ID NO:202):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC TGG ATG GGC TAG TAA ACT TGA-3'
DNA33087-p4 (SEQ ID NO:203):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA CCC TTC TGC TCC TTC TTG TT-3'

DNA34387 *(IS97-109)*:

The expression pattern of DNA34387 (SEQ ID NO:25) was observed in fetal and adult human tissues at the following sites:

Fetal - thyroid epithelium, small intestinal epithelium, gonad, pancreatic epithelium, hepatocytes in liver and renal tubules. Expression also seen in vascular tissue in developing long bones.

Adult - Moderate signal in placental cytotrophoblast, renal tubular epithelium, bladder epithelium, parathyroid and epithelial tumors.

The fetal (E12-E16 weeks) tissues examined included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb.

The adult human tissues examined: kidney (normal and end-stage), adrenal, myocardium, aorta, spleen, lymph node, gall bladder, pancreas, lung, skin, eye (inc. retina), prostate, bladder, liver (normal, cirrhotic, acute failure).

The non-human primate tissues examined included the following:

Chimp tissues: Salivary gland, stomach, thyroid, parathyroid, skin, thymus, ovary, lymph node.
Rhesus Monkey Tissues: Cerebral cortex, hippocampus, cerebellum, penis.

The probes used in this procedure were the following:

DNA34387-p1 (SEQ ID NO:206):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CCG AGA TAT GCA CCC AAT GTC-3'
DNA34387-p2 (SEQ ID NO:207):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA TCC CAG AAT CCC GAA GAA CA-3'

DNA35638:

IS97-078:

Expression of DNA35638 (SEQ ID NO:35) was observed in the endothelium lining a subset of fetal and placental vessels. Endothelial expression was confined to these tissue blocks. Expression also observed over intermediate trophoblast cells of placenta.

The fetal tissues examined (E12-E16 weeks) included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb.

The adult tissues examined included: liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung, skin, cerebral cortex (rm), hippocampus(rm), cerebellum(rm), penis, eye, bladder, stomach, gastric carcinoma, colon, colonic carcinoma, thyroid (chimp), parathyroid (chimp) ovary (chimp) and chondrosarcoma. Also examined was tissue derived from acetaminophen induced liver injury and hepatic cirrhosis.

The oligos used for the above procedure were the following:
DNA35638-p1 (SEQ ID NO:208):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GGG AAG ATG GCG AGG AGG AG-3'
DNA35638-p2 (SEQ ID NO:209):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA CCA AGG CCA CAA ACG GAA ATC-3'

DNA39523:

The following probes were used in the *in situ* studies below:
DNA39523-p1 (SEQ ID NO:210):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC AGC GCA CGG CCA CAG ACA-3'
DNA39523-p2 (SEQ ID NO:211):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GAC CCT GCG CTT CTC GTT CCA-3'

198-052:

DNA39523 (SEQ ID NO:45) in normal human skin (neonatal foreskin) and adult psoriatic skin both exhibited specific strong expression in the epithelial cells of the stratum basale - the single layer along the basement membrane which is the progenitor for all of the overlying epidermal cells in the skin.

There was no expression in epidermal cells in the overlying layers (stratum spinosum, straum granulosum, *etc.*). The intensity of the signal was slightly increased in psoriatic skin. Expression was also

apparent in the dermis (the connective tissue immediately underlying the epidermis) of both normal and psoriatic skin. Expression here was most apparent in spindle shape cells within the collagen matrix - the stromal fibroblasts.

In the brain, sections of cerebrum had strong specific expression in a subset of superficial cortical neurons - a distinct pattern suggestive of a specific population of cortex neurons.

In inflamed and normal bowel: Normal human large bowel and bowel with either Crohns's disease or ulcerative colitis had specific moderate to strong expression in a multifocal pattern within the lamina propria of villi. The cells labeled by *in situ* were spindloid stromal cells best delineated as fibroblasts. There was no expression by intestinal epithelial cells and there was no apparent increased expression (intensity or frequency) in diseased bowel. Specifically there was also no correlation of expression and lesions in the inflamed bowel.

In human fetal kidney, there was specific weak to moderate expression in multifocal developing tubules; expression was in the tubular epithelium in these foci.

The expression of DNA39523 (SEQ ID NO:45) in the skin and specific localization to the basal epithelial cells of the epidermis cells suggests a potential role in differentiation/maintenance of the basal epidermal cells. This expression pattern in combination with the fact that expression occurs in cells that are directly adjacent to the basement lamina, suggests that the cells regulate trafficking of leukocytes into the epidermis. As a result DNA39523 (SEQ ID NO:45) may be a constitutively expressed signal for the trafficking of dendritic/Langerhan cells or lymphocytes into the epidermis. Such trafficking is a normal physiologic event that occurs in normal skin and is thought to be involved in immunosurveillance of the skin.

The expression of DNA39523 (SEQ ID NO:45) in inflammatory bowel disease was not increased from normal tissue, and there was no correlation of its expression to inflammatory lesions. Similarly, its expression in the basal epidermal cells in psoriatic skin lesions was equivalent to or only slightly greater than that seen in normal neonatal skin (but age-matched control adult skin was not available at the time of the study).

IS97-128:

The expression of DNA39523 (SEQ ID NO:45) was observed in the epithelium of mouse embryo skin as well as the basal epithelium and dermis of human fetal skin. The basal epithelial pegs of the squamous mucosa of the chimp tongue are also positive. Expression was also observed in a subset of cells in developing glomeruli of fetal kidney, adult renal tubules, and over "thyroidized" epithelium in end-stage renal disease. However, low expression was also seen in a renal cell carcinoma, probably over the epithelial cells. Expression was also observed in the stromal cells both (1) at low levels in fetal lung, and (2) in the apical portion of gastric glands. High expression was indicated in the lamina propria of the fetal small intestinal villi, normal colonic mucosa and over stromal cells in a colonic carcinoma. Strong expression occurred in benign connective tissue cells in the hylanized stroma of a sarcoma. Expression also occurred in stromal cells in the placental villi and the splenic red pulp. In the brain, expression occurred in cortical neurones.

DNA39523 (SEQ ID NO:45) was also expressed in the connective tissue surrounding developing bones and over nerve sheath cells in the fetus.

The fetal tissues examined (E12-E16 weeks) included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb. The adult tissues examined included: liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung, skin, cerebral cortex (rm), hippocampus(rm), eye, stomach, gastric carcinoma, colon, colonic carcinoma, thyroid (chimp), parathyroid (chimp) ovary (chimp) and chondrosarcoma. Also examined included acetaminophen induced liver injury and hepatic cirrhosis.

IS98-092:

The expression of DNA39523 (SEQ ID NO:45) was present in many cells in the outer layers (I and II) of the monkey cerebral cortex. A small subset of cells in the deeper cortical layers also expressed mRNA for the chemokine homolog. Scattered cells within the molecular layers of the hippocampus and bordering the inner edge of the dentate gyrus showed expression of DNA39523 (SEQ ID NO:45). No expression was detected within the cerebellar cortex. Expression of DNA39523 (SEQ ID NO:45) was not observed in infarcted brain, where cell death has occurred in the regions where the chemokine homolog normally is expressed. DNA39523 (SEQ ID NO:45) could possibly serve as a marker of a subset of neurons of outer layers of the cerebral cortex and could possibly reveal neuronal migration disorders. Abnormal neuronal migration is a possible cause of some seizure disorders and schizophrenia.

IS98-128:

DNA39523 (SEQ ID NO:45) showed intriguing and specific patterns of hybridization within postnatal day (P)10 and adult mouse brains. In one sagittal section of P10 mouse brain, strong signal was observed scattered within the molecular layer of the hippocampus and inner edges of the dentate gyrus. Cells in the presubiculum were moderately labeled; the signal extended in a strong band through outer layers of the retrosplenial cortes to the occipital cortex, where the signal diminished to background levels. A small set of positive neurons were detected in deeper regions of P10 motor cortex; neurons in outer layers of P10 cortex did not exhibit signal above background levels. Moderate hybridization signal was also detected in the inferior colliculus. Chemokine homolog signal in the adult mouse brain was evaluated in three coronal sections at different levels. Strong signal was detected in the septum and in scattered neurons in the pontine nuclei and motor root of the trigeminal nerve; moderate signal was seen in the molecular layers of the hippocampus and outer layers of the retrosplenial cortex.

IS99-027:

Bolekine (also known as BRAK - the chemokine to which DNA39523 (SEQ ID NO:45) bear significant homology) belongs to a chemokine subgroup characterized by a cys-x-cys (CXC) motif and absence of an amino-terminal glu-leu-arg (ELR). Non-ELR CXC chemokines (includingSDF-1, IP10, Mig and PF4) are chemotactic for subsets of leukocytes including B and T lymphocytes. They also have angiostatic activity.

DNA39523 (SEQ ID NO:45) was detected in Postnatal day (P) 1 mouse brain, bolekine signal was detected in the hippocampus (stratum lacunosum moleculare and hilus of the dentate gyrus) and anterior

olfactory nucleus, but not in the developing cerebral cortex or cerebellum. By P10, signal is present in a subset of cells in layers 1 & 2 of the cerebral cortex. A small population of cells in the deeper layers also express DNA39523 (SEQ IDNO:45). The pattern in the hippocampus resembled the P1 brain. Weak signal is present in the cerebellum, especially lobules IX and X. Signal is also present in the dorsal striatum and colliculi.

In the adult mouse brain, bolekine-positive cells were difficult to detect in the adult cerebral cortex, but signal is present in the anterior olfactory nucleus and hippocampus. In ischemic mouse brains, however, bolekine signal is induced in the penumbra.

In the developing cerebral cortex, bolekine expression correlates with final stages of neuronal migration and the establishment of axonal projections and synaptogenesis. Other CXC chemokines have roles in neuronal migration and patterning in the central nervous system (SDF-1) and modulation of neuronal activity (IL-8 and GRO-a).

Bolekine expression is induced in ischemic -reperfusion injury in the brain, but not in other inflammatory states.

DNA47365 *(IS97-142)*: In fetal tissues, the expression of DNA47635 (SEQ ID NO:91) was observed in the fascia lining the anterior surface of the vertebral body. There is expression over the fetal retina. Low level expression over fetal neurones.

The following probes were used in the above analysis:

DNA47365-p1 (SEQ ID NO:214):

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC AAC CCG AGC ATG GCA CAG CAC-3'

DNA47365-p2 (SEQ ID NO:215):

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA TCT CCC AGC CGC CCC TTC TC-3'

DNA49435 *(IS97-136)*:

Moderate expression of DNA49435 (SEQ ID NO:111) was observed over cortical neurones in the fetal brain. Expression was also present over the inner aspect of the fetal retina, possible expression in the developing lens. Expression was seen over fetal skin, cartilage, small intestine, placental villi and umbilical cord. In adult tissues there is an extremely high level of expression over the gallbladder epithelium. Moderate expression of DNA49435 (SEEQ ID NO:111) was seen over the adult kidney, gastric and colonic epithelia.

The human fetal tissues examined (E12-E16 weeks) included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis, testis and lower limb. The adult human tissues examined included: kidney (normal and end-stage), adrenal, spleen, lymph node, pancreas, lung, eye (inc. retina), bladder, liver (normal, cirrhotic, acute failure).

The non-human primate tissues examined included the adrenal glands from chimp tissues and the cerebral cortex, hippocampus and cerebellum of rhesus monkey tissues.

The probes used in the above analysis were the following:

DNA49435-p1 (SEQ ID NO:218):

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GGA TCC TGG CCG GCC TCT G-3'

DNA49435-p2 (SEQ ID NO:219):

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GCC CGG GCA TGG TCT CAG TTA-3'

DNA54228 *(IS98-105)*:

Expression of DNA54228 (SEQ ID NO:133) was observed in bone spicules: fetal metaphyseal bone, fetal calvarium (skull) and bone tissue in human neoplasia (osteosarcoma and chondrosarcoma). There is weak but consistent signal in small bone spicules in the metaphysis of fetal bone and in ossified spicules in a chondrosarcoma and an osteosarcoma. No signal was detected in human lung, liver, thymus, kidney, thyroid, brain, spleen, fetal tissues including adrenal, brain, cartilage, lung, liver, intestine, gonad, heart and skin.

The probes used in the above procedure were the following:

hmDETI-p1 (SEQ ID NO:220):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC ACC ACC ACC CAG GAG C-3'
hmDETI-p2 (SEQ IDNO:221):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA AAT GAA GTG GGA CGT TTG AGT-3'
DNA54228-p1 (SEQ ID NO:222):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CTT CTT TCC TTC ACC ACC ACC-3'
DNA54228-p2 (SEQ ID NO:223):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA TCT GCC TTG GCT TTT GAC AC-3'

DNA54231 (mFIZZ3):

IS98-070:

DNA54231 (SEQ ID NO:139) showed a moderate signal that is specific to adipocytes. This signal was present in mesenteric fat and in interstitial fat in the neck around the trachea. The expression pattern appears to be specific for adult fat.

IS98-109:

The expression of DNA54231 (SEQ ID NO:139) was specific to adipocytes and was present wherever such cells were found which in this study included the peritoneal mesentery, perirenal fat in the renal pelvis, and the mammary fat pad. There was no expression in any other cell type in normal murine brain, liver, kidney, mammary gland, pancreas, spleen, pancreas, bone marrow, stomach, duodenum, jejunum, ileum, colon, cecum, testis, skin, or lung.

The selective distribution of this molecule to adipocytes suggests a role in either fat metabolism or the production/genesis of adipocytes, either of which is important in obesity.

The probes used for the above procedure were the following:

DNA54231-p1 (SEQ ID NO:224):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CGA GGG GGA CAG GAG CTA ATA-3'
DNA54231-p2 (SEQ ID NO:225):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GTC CCA CGA GCC ACA GG-3'

DNA59294 *(IS98-138)*:

DNA 59294 (SEQ ID NO:149) was evaluated in a panel consisting of normal adult and fetal tissues and tissues with inflammation, predominantly chronic lymphocytic inflammation. In summary, the expression was specific to muscle, certain types of smooth muscle in the adult and in skeletal and smooth muscle in the human fetus. The expression in adult human was in smooth muscle of tubular organs evaluated including colon and gall bladder. There was no expression in the smooth muscle of vessels or bronchi. No adult human skeletal muscle was evaluated. In fetal tissues there was moderate to high diffuse expression in skeletal muscle the axial skeleton and limbs. There was weak expression in the smooth muscle of the intestinal wall but no expression in cardiac muscle.

In adult tissues, the colon showed a low level of diffuse expression in the smooth muscle (tunica muscularis) in 5 specimens with chronic inflammatory bowel disease. In the gall bladder, there was weak to low level expression in the smooth muscle of the gall bladder.

In fetal human tissues, there was moderate diffuse expression in skeletal muscle and weak to low expression in smooth muscle. However expression was not detected in the fetal heart or any other fetal organ including liver, spleen, CNS, kidney, gut, lung.

The additional human tissues tested with no detectable expression included: lung with chronic granulomatous inflammation and chronic bronchitis (5 patients), peripheral nerve, prostate, heart, placenta, liver (disease multiblock including acetomihopin induced injury and cirrhosis), brain (cerebrum and cerebellum), tonsil (reactive hyperplasia), peripheral lymph node, thymus.

The probes used in the above procedure were the following:

626.p1 (SEQ ID NO:226):
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CGG AAT GGA CTG GCC TCA CAA-3'
626.p2 (SEQ ID NO:227):
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA AGG ATG GTC TCG GGC TGC TG-3'

DNA30868 *(IS97-044)*

DNA30868 expression was found in the following fetal tissues: spinal cord, autonomic ganglia, enteric nerves, sacral plexus, peripheral and cranial nerves.

The fetal tissues examined were the following: Placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb.

The adult tissues examined included: Liver, kidney, adrenal, myocardium, aorta, spleen, lymph node, pancreas, lung and skin.

The probes used for the above procedure were the following:

DNA30868.p1 (C111-G): (SEQ ID NO:304)
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC AGA GAC AGG GCA AGC AGA ATG-3'
DNA30868.p2 (C111-H): (SEQ ID NO:305)
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GAA GGG GAT GAC TGG AGG AAC-3'

DNA53517:

IS98-070:

DNA53517 (SEQ ID NO:255) expression in the normal adult murine lung was patchy, with expression in a subset of mucosal epithelial cell in the large airway (bronchi/bronchioles). There is also expression within the rare discrete cells in the submucosal interstitium adjacent to the large airways. These cells, typically 1-3 within a positive focus, are adjacent to large vessels and may represent smooth muscle cells, peripheral nerves or Schwann cells, or lymphatics.

In the murine adult lung with allergic inflammation (eosinophilic, lymphocytic vasculitis, bronchiolitis and pneumonitis), there was diffuse strong expression in all mucosal epithelial cells of all of the large airways (bronchi/bronchioles) of the lung. There was also strong expression in discrete cells that represent a subset of epithelial cells that line the alveoli; these cells are type II pneumocytes. There is also expression, as in normal lung, present within rare discrete cells in the submucosal interstitium adjacent to the large airways.

In normal adult murine small and large intestine, there is strong expression within multifocal few discrete single cells that are present in the submucosa, the tunica muscularis and the mesentery. The cells that express the signal are almost always associated with nerve, vein, artery triads within these areas. These cells are spindle shaped and may be either a peripheral nerves, Schwann cells associated with such nerves or some type of support cell associated with vessel or lymphatics. Interestingly, there is no expression within identifiable myenteric plexi that are present within the tunica muscularis.

In inflamed large bowel (from an IL10R KO mouse) the pattern of expression is similar but expression level is significantly decreased.

IS98-093:

The distribution of DNA53715 (SEQ ID NO:255) was further evaluated in a broad screen of normal murine tissues. In normal lung, expression is variable but when present was restricted to murine bronchial epithelial cells and type II alveolar cells in the lung. There is a marked increase in expression in these cells in inflamed lung (allergic inflammation with bronchial mucosal hypertrophy/hyperplasia; asthma model). The expression of DNA53715 (SEQ ID NO:255) in the bowel is most prominent in the colon and is present in few discrete cells within the submucosa and mucosa muscularis, the thin, well vascularized tissue layer between the muscle wall of the bowel and the mucosa proper. The exact identity of these cells has not been delineated, however, their spindloid morphology and close association to capillaries and small vessels in the submucosa suggest the following possibilities: a subset of vascular pericytes or non-myelinated nerve fibers.

The expression of DNA53715 (SEQ ID NO:255) in discrete cells in the bowel submucosa was restricted to the colon and was not seen in sections of jejunum, ileum, proximal duodenum or stomach. Expression was not detected in the following normal murine tissues: liver, kidney, spleen, bone marrow, lung, pancreas, stomach, proximal duodenum, jejunum, ileum, brain, skin, testis, or mammary glands.

It is possible that DNA53715 (SEQ ID NO:255) has a role in enhancing or stimulating mucosal immunity in the lung.

The probes used for the above procedure were the following:

DNA53517.p1 (C301-P): (SEQ ID NO:308)

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CCC AGG ATG CCA ACT TTG A-3'

DNA53517.p2 (C301-Q): (SEQ ID NO:309)

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA AGG AGG CCC ATC TGT TCA TAG-3'

EXAMPLE 7

In situ Hybridization in Cells and Diseased Tissues

The *in situ* hybridization method of Example 6 is used to determine gene expression, analyze the tissue distribution of transcription, and follow changes in specific mRNA synthesis for the genes/DNAs and the proteins of the invention in diseased tissues isolated from human individuals suffering from a specific disease. These results show more specifically where in diseased tissues the genes of the invention are expressed and are more predictive of the particular localization of the therapeutic effect of the inhibitory or stimulatory compounds of the invention (and agonists or antagonists thereof) in a disease. Hybridization is performed according to the method of Example 6 using one or more of the following tissue and cell samples:

(a) lymphocytes and antigen presenting cells (dendritic cells, Langherhans cells, macrophages and monocytes, NK cells);

(b) lymphoid tissues: normal and reactive lymph node, thymus, Bronchial Associated Lymphoid Tissues, (BALT), Mucosal Associated Lymphoid Tissues (MALT);

(c) human disease tissues:

- Synovium and joint of patients with Arthritis and Degenerative Joint Disease;
- Colon from patients with Inflammatory Bowel Disease including Ulcerative Colitis and Crohns' disease;
- Skin lesions from Psoriasis and other forms of dermatitis;
- Lung tissue including BALT and tissue lymph nodes from chronic and acute bronchitis, pneumonia, pneumonitis, pleuritis;
- Lung tissue including BALT and tissue lymph nodes from Asthma;
- nasal and sinus tissue from patients with rhinitis or sinusitis;
- Brain and Spinal cord from Multiple Sclerosis, Alzheimer's Disease and Stroke;
- Kidney from Nephritis, Glomerulonephritis and Systemic Lupus Erythematosis;
- Liver from Infectious and non-infectious Hepatitis and acetaminophen-induced liver cirrhosis;
- Tissues from Neoplasms/Cancer.

Expression is observed in one or more cell or tissue samples indicating localization of the therapeutic effect of the compounds of the invention (and agonists or antagonists thereof) in the disease associated with the cell or tissue sample.

The sequences of the oligonucleotides used, where expression overlaps with the non-diseased tissue distribution reported earlier is recited in Example 6.

DNA30942:

IS98-021: Expression was observed in mononuclear phagocytes in the normal chimp thymus, as well as in a gastric carcinoma (1/1) colorectal cancer (1/1), breast cancer (2/5) and a lung cancer (1/4). Expressed by malignant cells in an osteosarcoma and a poorly differentiated liposarcoma. Possible signal in the malignant cells of a testicular teratoma and breast cancers (1/5). In one of the lung cancers scattered signal is seen over a

high endothelial venule within pulmonary lymphoid tissue. The fetal tissues examined (E12-E16 weeks) included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb. The adult human tissues examined included: liver, kidney, adrenal, myocardium, aorta, spleen, lung, skin, chondrosarcoma, eye, stomach, gastric carcinoma, colon, colonic carcinoma, renal cell carcinoma, prostate, bladder mucosa and gall bladder. Also examined was tissue derived from acetaminophen induced liver injury and hepatic cirrhosis. The rhesus tissues examined include: cerebral cortex (rm), hippocampus(rm). The chimp tissues examined included: thyroid, parathyroid, ovary, nerve, tongue, thymus, adrenal, gastric mucosa and salivary gland.

IS98-085: Expression was observed in eight adenocarcinomas and seven squamous lung carcinomas. Actins were strongly positive in all tumors, indicating that all are suitable for *in situ* hybridization analysis. Expression of DNA30942 was observed in 6 of the tumors as follows:

6727-95 / squamous carcinoma - Strongly expressed over neoplastic epithelium;

9558-95 / squamous carcinoma - Expression over neoplastic epithelium;

12235-95 / adenocarcinoma - Expression over *in situ* and infiltrating tumor cells;

6545-95 & 4187-96 / squamous carcinomas - Expression over cells in tumor stroma, no expression seen over tumor cells;

12954-94 / squamous carcinoma - possible weak expression over stromal cells.

IS99-112:

The *in situ* expression of DNA30942 (SEQ ID NO:13) was evaluated numerous chronic inflammatory conditions and lymphoid organs. In summary, DNA30942 (SEQ ID NO:13) was strongly expressed in high endothelial venules (HEV) in the tonsil, hilar lymph node, bronchial mucosal-associated lymphoid tissue (BALT) in chronic asthma, patchy expression in colonic mucosa and weak inconsistent expression in gut-mucosal associated lymphoid tissues (GALT) HEV.

In lymphoid tissues, there was observed strong specific expression in single sections of tonsil, hilar lymph node, bronchial mucosal-associated lymphoid tissue BALT) in a case of chronic asthma, and in gut mucosal associated lymphoid tissues in sections of IBD (GALT/MALT). In each of these lymphoid organs expression specifically was present in high-endothelial venules (HEV).

In tissue in a chronic asthmatic lung, additionally to expression in BALT HEVs, specific expression was observed in small capillaries lined with high or reactive swollen endothelial cells in the submucosa of inflamed bronchi. This region was not intimately associated with BALT but was specific to the submucosal site for inflammatory cell trafficking to the bronchi. There was a significant submucosal infiltrate of eosinophils in these areas. In other sections of diseased lung (COPD and chronic interstitial pneumonia) there was not any expression of DNA30942 (SEQ ID NO:13), these sections had some artifact (loss of tissue from slide).

In psoriatic tissue, there was weak expression in some small dermal capillaries in psoriatic plaques. In tonsilar tissue, additional to expression in HEVs associated with follicles, there was also strong expression within the reticulated tonsillar crypt epithelium. Expression here was also in vessels in the small intra-epithelial

capillaries present. Expression was also within some of the epithelial cells. This is an important immunological site and is involved with antigen presentation and may play a role in tolerance induction.

In tissue isolated from patients suffering from Crohns' Disease and ulcerative colitis, colonal expression was present in the mucosa with patchy distribution in some but not in all cases. Expression in HEV in GALT was present as a significantly weaker signal than seen in other lymphoid tissues and was not consistently present even in sections where there was strong but patchy expression in the mucosa.

In tissue isolated from acetaminophen induced liver injury and cirrhosis, there was weak expression in small capillaries within areas in the portal tracts with chronic lymphocytic inflammation.

DNA33460 *(IS98-015)*:

The expression of DNA33460 (SEQ ID NO:20) was observed over cells in loose connective tissue immediately adjacent to developing extra ocular muscle in the fetal eye. Moderate expression over soft-tissue sarcoma. The fetal tissues examined (E12-E16 weeks) included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis and lower limb. The adult tissues examined included the liver, kidney, renal cell carcinoma, adrenal, aorta, spleen, lymph node, pancreas, lung, myocardium, skin, cerebral cortex (rm), hippocampus (rm), cerebellum (rm), bladder, prostate, stomach, gastric carcinoma, colon, colonic carcinoma, thyroid (chimp), parathyroid (chimp) ovary (chimp) and chondrosarcoma. Also examined was tissue extracted from acetaminophen induced liver injury and hepatic cirrhosis.

The probes used in this procedure were the following:

DNA33460-p1 (SEQ ID NO:204):

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CAG CAC TGC CGG GAT GTC AAC-3'

DNA33460-p2 (SEQ ID NO:205):

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GTT TGG GCC TCG GAG CAC TG-3'

DNA34387 *(IS98-083)*:

Expression observed in lung cancer tumors and was positive in all eight squamous carcinomas and in 6/8 adenocarcinomas. Expression levels are low to moderate in the adenocarcinomas and very strong in the squamous carcinomas. No expression was seen in the tumor stroma, alveoli or normal respiratory epithelium. Possible low level expression in the lymph nodes.

Expression was observed in lung cancer. The gene was amplified in Taqman analysis of a lung tumor panel. Expression was observed in eight squamous carcinomas and in 6/8 adenocarcinomas. Expression was seen in *in situ* and infiltrating components. Expression levels were low to moderate in the adenocarcinomas. In general expression was higher in the squamous carcinomas and in two the expression was strong. Possible low level expression in lymph nodes.

DNA35638:

IS98-124:

This study examined the expression of DNA35638 (SEQ ID NO:35) in inflamed human tissues (psoriasis, IBD, inflamed kidney, inflamed lung, hepatitis (liver block), normal tonsil, adult and chimp

multiblocks) DNA35638 (SEQ ID NO:35) has been shown elsewhere in this application to have immunostimulatory (enhances T lymphocyte proliferation in the MLR and costimulation) and proinflammatory properties (induces a neutrophil infiltrate *in vivo*).

This study evaluated the differential expression of this molecule in vessels of inflamed human tissues as compared to non-inflamed tissues. In summary, expression was present in the endothelium/intima of large vessels in the lung afflicted with chronic inflammation, in the superficial dermal vessels of the psoriatic skin, in arterioles in a specimen of chronic sclerosing nephritis, and in capillaries including the perifollucular sinuses of tonsil. DNA35638 (SEQ ID NO:35) was not expressed (as detectable by this methodology) in normal skin (human foreskin specimens), normal lung, inflamed (8 IBD specimens) or normal large bowel, chronically inflamed or cirrhotic liver, normal adult cardiac tissue, or adrenal gland.

DNA39523:

198-052:

DNA39523 (SEQ ID NO:45) in normal human skin (neonatal foreskin) and adult psoriatic skin both exhibited specific strong expression in the epithelial cells of the stratum basale - the single layer along the basement membrane which is the progenitor for all of the overlying epidermal cells in the skin.

There was no expression in epidermal cells in the overlying layers (stratum spinosum, straum granulosum, *etc.*). The intensity of the signal was slightly increased in psoriatic skin. Expression was also apparent in the dermis (the connective tissue immediately underlying the epidermis) of both normal and psoriatic skin. Expression here was most apparent in spindle shape cells within the collagen matrix - the stromal fibroblasts.

In inflamed and normal bowel: Normal human large bowel and bowel with either Crohns's disease or ulcerative colitis had specific moderate to strong expression in a multifocal pattern within the lamina propria of villi. The cells labeled by *in situ* were spindloid stromal cells best delineated as fibroblasts. There was no expression by intestinal epithelial cells and there was no apparent increased expression (intensity or frequency) in diseased bowel. Specifically there was also no correlation of expression and lesions in the inflamed bowel.

The expression of DNA39523 (SEQ ID NO:45) in the skin and specific localization to the basal epithelial cells of the epidermis cells suggests a potential role in differentiation/maintenance of the basal epidermal cells. This expression pattern in combination with the fact that expression occurs in cells that are directly adjacent to the basement lamina, suggests that the cells regulate trafficking of leukocytes into the epidermis. As a result DNA39523 (SEQ ID NO:45) may be a constitutively expressed signal for the trafficking of dendritic/Langerhan cells or lymphocytes into the epidermis. Such trafficking is a normal physiologic event that occurs in normal skin and is thought to be involved in immunosurveillance of the skin.

The expression of DNA39523 (SEQ ID NO:45) in inflammatory bowel disease was not increased from normal tissue, and there was no correlation of its expression to inflammatory lesions. Similarly, its expression in the basal epidermal cells in psoriatic skin lesions was equivalent to or only slightly greater than that seen in normal neonatal skin (but age-matched control adult skin was not available at the time of the study).

DNA45416 *(IS98-140)*:

The expression of DNA45416 (SEQ ID NO:79) was evaluated in a variety of human and non-human primate tissues and was found to be highly specific. Expression was present only in alveolar macrophages in the lung and in Kupffer cells of the hepatic sinusoids. Expression in these cells was significantly increased when these distinct cell populations were activated. Though these two subpopulations of tissue macrophages are located in different organs, they have similar biological functions. Both types of these phagocytes act as biological filters to remove material from the blood stream or airways including pathogens, senescent cells and proteins and both are capable of secreting a wide variety of important proinflammatory cytokines.

In inflamed lung (7 patient samples) expression was prominent in reactive alveolar macrophage cell populations defined as large, pale often vacuolated cells present singly or in aggregates within alveoli and was weak to negative in normal, non-reactive macrophages (single scattered cells of normal size). Expression in alveolar macrophages was increased during inflammation when these cells were both increased in numbers and size (activated). Despite the presence of histocytes in areas of interstitial inflammation and peribronchial lymphoid hyperplasia in these tissues, expression was restricted to alveolar macrophages. Many of the inflamed lungs also had some degree of suppurative inflammation: expression was not present in neutrophilic granulocytes.

In liver, there was strong expression in reactive/activated Kupffer cells in livers with acute centrilobular necrosis (acetaminophen toxicity) or fairly marked periportal inflammation. However there was weak or no expression in Kupffer cells in normal liver or in liver with only mild inflammation or mild to moderate lobular hyperplasia/hypertrophy. Thus, as in the lung, there was increased expression in acivated/reactive cells.

There was no expression of this molecule in histiocytes/macrophages present in inflamed bowel, hyperplastic/reactive tonsil or normal lymph node. The lack of expression in these tissues which all contained histiocytic inflammation or resident macrophage populations strongly supports restricted expression to the unique macrophage subset populations defined as alveolar macrophage and hepatic Kupffer cells. However, the expression of DNA454216 (SEQ ID NO:79) spleen or bone marrow was not available for evaluation.

Human tissues evaluated which had no detectable expression included: Inflammatory Bowel disease (7 patient samples with moderate to severe disease), tonsil with reactive hyperplasia, peripheral lymph node, psoriatic skin (2 patient samples with mild to moderate disease), heart, peripheral nerve. Chimp tissues evaluated which had no detectable expression included: tongue, stomach, thymus.

The probes used for the above studies were the following:

628.p1 (SEQ ID NO:212):

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CTC CAA GCC CAC AGT GAC AA-3'

628.p2 (SEQ ID NO:213):

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA CCT CCA CAT TTC CTG CCA GTA-3'

DNA41374:

IS-98-077:

DNA41374 (SEQ ID NO:248) was expressed in thymic T lymphocytes

Summary: In numerous tissues evaluated there expression was only detected as weak diffuse expression in thymic T lymphocytes. The limited distribution pattern suggests expression by T lymphocytes or cells closely associated with T lymphocytes such as antigen presenting cells (dendritic cell populations, *etc*). In inflamed human tissue with significant lymphocytic inflammation and presence of reactive follicle formation (inflammatory bowel disease and chronic lymphocytic interstitial pneumonia/bronchitis) there was no detectable expression in areas which contained significant numbers of T lymphocytes. The tissues tested for which there was no detectable expression included: human normal tissues: placenta, lung, spleen, adrenal gland, skin, kidney, eye, liver; human diseased tissue: liver disease: chronic hepatitis, chronic cholangitis, acute centrilobular necrosis (acetaminophen toxicity); Neoplasia (tumor multiblock): osteosarcoma, squamous cell carcinoma; human fetal tissues: brain, spinal cord, lung, heart, kidney, axial and limb musculoskeleton vessels, umbilical cord; non-human primate: tongue, thyroid gland, parathyroid gland, stomach, salivary gland.

IS98-125.

DNA41374 (SEQ ID NO:248) has low level expression in non-human primate thymus and in human tonsil in T lymphocyte specific regions. The limited distribution pattern suggests expression by T lymphocytes or cells closely associated with T lymphocytes such as antigen presenting cells (dendritic cell populations, *etc*). In inflamed tissue with significant lymphocytic inflammation and presence of reactive follicle formation (inflammatory bowel disease and chronic lymphocytic interstitial pneumonia/bronchitis) there was no detectable expression in areas which likely contain significant numbers of T lymphocytes.

Inflamed lung: (chronic lymphocytic and granulomatous pneumonitis): weak to negative signal in the interstitium compared to the control sense probe. There was weak expression in normal chimp thymus (human thymus not available) and in human tonsil. In the latter the expression was predominantly in T lymphocyte areas of this structure including the perifollicular marginal zone and in the paracortex.

There was no detectable expression in the following human tissues: inflammatory bowel disease (8 patient specimens), chronically inflamed and normal lung (6 patient specimens), chronic sclerosing nephritis (1), chronically and acutely inflamed and cirrhotic liver (10 specimen multiblock), normal and psoriatic skin, peripheral lymph node (non-reactive).

The probes used for the above procedures were the following:

41374.p1 (C337-G): (SEQ ID NO:306)
5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CTC CAC AGA ACC TCG CCA TCA-3'
41374.p2 (C337-H): (SEQ ID NO:307)
5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA TGG GGC AAG ACT CAC AAG CAG-3'

DNA53517:

IS98-070:

DNA53517 (SEQ ID NO:255) expression in the normal adult murine lung was patchy, with expression in a subset of mucosal epithelial cell in the large airway (bronchi/bronchioles). There is also expression within the rare discrete cells in the submucosal interstitium adjacent to the large airways. These

cells, typically 1-3 within a positive focus, are adjacent to large vessels and may represent smooth muscle cells, peripheral nerves or Schwann cells, or lymphatics.

In the murine adult lung with allergic inflammation (eosinophilic, lymphocytic vasculitis,, bronchiolitis and pneumonitis), there was diffuse strong expression in all mucosal epithelial cells of all of the large airways (bronchi/bronchioles) of the lung. There was also strong expression in discrete cells that represent a subset of epithelial cells that line the alveoli; these cells are type II pneumocytes. There is also expression, as in normal lung, present within rare discrete cells in the submucosal interstitium adjacent to the large airways.

In normal adult murine small and large intestine, there is strong expression within multifocal few discrete single cells that are present in the submucosa, the tunica muscularis and the mesentery. The cells that express the signal are almost always associated with nerve, vein, artery triads within these areas. These cells are spindle shaped and may be either a peripheral nerves, Schwann cells
associated with such nerves or some type of support cell associated with vessel or lymphatics. Interestingly, there is no expression within identifiable myenteric plexi that are present within the tunica muscularis.

In inflamed large bowel (from an IL10R KO mouse) the pattern of expression is similar but expression level is significantly decreased.

IS98-135:

DNA53715 (SEQ ID NO:255, mouse FIZZ-1) was used as a detection probe in the following human tissues: gastric carcinoma, inflamed lung (3 patients) (vessels, alveoli, large airways and mucous glands), aorta, heart, placenta and gall bladder.

Expression of mouse DNA53715 (SEQ ID NO:255) was present in normal mouse lung in large airway epithelium and had marked increased expression in inflamed murine lung (airway epithelium, type II alveolar pneumocytes). It was also expressed in discrete cells in the submucosa of the large bowel along vascular channels.

DNA84210:

The following probes were used in the *in situ* studies indicated below:

84210.p1 (F-79619): (SEQ ID NO:310)

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GCG GTC GCA GGA CAT TCA GTA-3'

84210.p2 (F-79620): (SEQ ID NO:311)

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA ACT CTT TGG GTT CCA GCA CAC-3'

DNA84210 (SEQ ID NO:285) is expressed in fetal kidney, primarily in developing glomeruli and tubules of the cortical zone and also weakly in fetal lung and spinal cord. There is also expression in stromal cells adjacent to developing cartilage and bone. In adult tissues, weak expression is seen in normal bronchial epithelium, in one (adenocarcinoma) of five lung tumors (2 squamous and 3 adenocarcinomas) and in a chondrosarcoma. There is possibly expression in the skin and its appendages, however, the section is folded and difficult to evaluate.

IS99-102:

Expression of DNA84210 (SEQ ID NO:285) in malignant melanoma, lung tumor, colon tumor, cell pellet, mouse tissues, fetal tissues.

Expression of DNA84210 (SEQ ID NO:285) is seen in several adult (neoplastic and non-neoplastic) and fetal tissues. As far as normal adult tissues are concerned, DNA84210 (SEQ ID NO:285) is seen in the epidermis of skin (mostly in basally located cells) and in skin appendages, such as hair follicles and sebaceous glands associated with them. Expression is also seen in bronchial epithelium and submucosal bronchial glands. In human fetal tissues, expression of DNA84210 (SEQ ID NO:285) is seen in skin and skin appendages, lung, renal cortex and pancreatic ducts. It is also seen in mesenchymal cells adjacent to developing bone and cartilage. There is no hybridization signal seen in mouse embryos. Expression of DNA84210 (SEQ ID NO:285) is seen in one of six colorectal adenocarcinomas (weak), 2 of 3 lung adenocarcinomas (one shows strong, but very focal expression, one is very weakly positive), 0 of 3 lung squamous cell carcinomas and 1 of 1 chondrosarcomas (weak). Expression is also seen in 5 of 5 malignant melanomas, the intensity of expression ranges from very weak to strong. These sections also demonstrate expression of DNA84210 (SEQ ID NO:285) in normal epidermis and skin appendages.

EXAMPLE 8

Use of the PRO polypeptides as a hybridization probe

The following method describes use of a nucleotide sequence encoding the PRO polypeptides as a hybridization probe.

DNA comprising the coding sequence of full-length or mature PRO polypeptides is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO - derived probe (*e.g*, PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727) to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO polypeptide can then be identified using standard techniques known in the art.

EXAMPLE 9

Expression of the PRO polypeptide in *E. coli*

This example illustrates preparation of an unglycosylated form of the PRO polypeptides by

recombinant expression in *E. coli*.

The DNA sequence encoding the PRO polypeptide is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar *et al.*, *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO polypeptide coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*; *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO polypeptide protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

The PRO polypeptides may also be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding a PRO polypeptide is initially amplified using selected PCR primers. The primers contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences are then ligated into an expression vector, which is used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 is reached. Cultures are then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g $(NH_4)_2SO_4$, 0.71 g sodium citrate·2H2O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM $MgSO_4$) and grown for approximately 20-30 hours at 30°C with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets are frozen until purification and refolding.

E. coli paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify.

Depending on condition the clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein was pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4°C for 12-36 hours. The refolding reaction is quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide gels and fractions containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded PRO polypeptide proteins are pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

EXAMPLE 10

Expression of the PRO polypeptides in mammalian cells

This example illustrates preparation of a potentially glycosylated form of the PRO polypeptide in recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the respective PRO DNA using ligation methods such as described in Sambrook *et al.*, *supra*. The resulting vector is called, for example, pRK5-PRO.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg of the pRK5-PRO DNA is mixed with about 1 µg DNA encoding the VARNA gene [Thimmappaya *et al.*, *Cell*, 31:543 (1982)] and dissolved in 500 uL of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M $CaCl_2$. To this mixture is added, dropwise, 500 µL of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM $NaPO_4$, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The

culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 uCi/ml ^{35}S-cysteine and 200 uCi/ml ^{35}S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of the polypeptide of the invention polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, pRK5-PRO may be introduced into 293 cells transiently using the dextran sulfate method described by Somparyrac *et al.*, *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-PRO is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing the expressed polypeptide of the invention can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, the polypeptides of the invention can be expressed in CHO cells. The pRK5-PRO can be transfected into CHO cells using known reagents such as $CaPO_4$ or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ^{35}S-methionine. After determining the presence of a polypeptide of the invention polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed polypeptide of the invention can then be concentrated and purified by any selected method.

Epitope-tagged polypeptide of the invention may also be expressed in host CHO cells. The DNA encoding the desired polypeptide of the invention may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged polypeptide of the invention insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged polypeptide of the invention can then be concentrated and purified by any selected method, such as by Ni^{2+}-chelate affinity chromatography.

EXAMPLE 11

Expression of PRO in Yeast

The following method describes recombinant expression of PRO in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of the PRO polypeptide from the ADH2/GAPDH promoter. DNA encoding a polypeptide of the invention and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of the PRO. For secretion, DNA encoding the PRO can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native sequence PRO signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of the polypeptide of the invention.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing the polypeptide of the invention may further be purified using selected column chromatography resins.

EXAMPLE 12

Expression of PRO in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO in Baculovirus-infected insect cells.

The sequence coding for PRO is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding a polypeptide of the invention or the desired portion of the coding sequence of the DNA encoding a PRO polypeptide [such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular] is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley *et al.*, *Baculovirus expression vectors: A Laboratory Manual*, Oxford: Oxford University Press (1994).

Expressed poly-his tagged polypeptide of the invention can then be purified, for example, by Ni^{2+}-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert *et al.*, *Nature*, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in

sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM $MgCl_2$; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 um filter. A Ni^{2+}-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A_{280} with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A_{280} baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni^{2+}-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His_{10}-tagged- polypeptide of the invention are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO polypeptide can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

EXAMPLE 13

Preparation of Antibodies that Bind PRO

This example illustrates preparation of monoclonal antibodies which can specifically bind the polypeptides of the invention.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include the purified polypeptide of the invention itself, fusion proteins containing the respective polypeptide of the invention, and cells expressing recombinant polypeptide of the invention on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the polypeptide of the invention immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect antibodies specific to the respective polypeptide of the invention.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of the respective polypeptide of the invention. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells are screened in an ELISA for reactivity against the respective polypeptide of the invention. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against the polypeptides of the invention is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

Material	UNQ	PRO	ATCC #	ATCC Deposit Date
DNA29101-1276	174	200	209653	March 5, 1998
DNA30871-1157	178	204	209380	October 16, 1997
DNA30942-1134	186	212	209254	September 16, 1997
DNA33087-1158	190	216	209381	October 16, 1997
DNA33460-1166	200	226	209376	October 16, 1997
DNA34387-1133	214	240	209260	September 16, 1997
DNA35558-1167	209	235	209374	October 16, 1997
DNA35638-1141	219	245	209265	September 16, 1997
DNA35916-1161	146	172	209419	October 28, 1997
DNA39523-1192	240	273	209424	October 31, 1997
DNA40620-1183	239	272	209388	October 17, 1997
DNA40982-1235	293	332	209433	November 17, 1997
DNA44184-1319	330	526	209704	March 26, 1998
DNA44205-1285	365	701	209720	March 31, 1998
DNA45410-1250	316	361	209621	February 5, 1998
DNA45416-1251	317	362	209620	February 5, 1998
DNA45419-1252	318	363	209616	February 5, 1998

	DNA47365-1206	319	364	209436	November 7, 1997
	DNA47470-1130	313	356	209422	October 28, 1997
	DNA48314-1320	332	531	209702	March 26, 1998
	DNA49435-1219	334	533	209480	November 21, 1997
5	DNA50921-1458	540	1083	209859	May 12, 1998
	DNA53974-1401	434	865	209774	April 14, 1998
	DNA54228-1366	408	770	209801	April 23, 1998
	DNA54231-1366	407	769	209802	April 23, 1998
	DNA56405-1357	430	788	209849	May 6, 1998
10	DNA57033-1403	557	1114	209905	May 27, 1998
	DNA57690-1374	491	1007	209950	June 9, 1998
	DNA59220-1514	598	1184	209962	June 9, 1998
	DNA59294-1381	516	1031	209866	May 14, 1998
	DNA59776-1600	701	1346	203128	August 18, 1998
15	DNA59849-1504	585	1155	209986	June 16, 1998
	DNA60775-1532	633	1250	203173	September 1, 1998
	DNA61873-1574	678	1312	203132	August 18, 1998
	DNA62814-1521	606	1192	203093	August 4, 1998
	DNA64885-1529	630	1246	203457	November 3, 1998
20	DNA65404-1551	653	1283	203244	September 9, 1998
	DNA65412-1523	608	1195	203094	August 4, 1998
	DNA66675-1587	698	1343	203282	September 22, 1998
	DNA68864-1629	732	1418	203276	September 22, 1998
	DNA68872-1620	722	1387	203160	August 25, 1998
25	DNA68874-1622	728	1410	203277	September 22, 1998
	DNA76400-2528	900	1917	203573	January 12, 1999
	DNA77624-2515	859	1868	203553	December 22, 1998
	DNA30868-1156	179	205	------	March 2, 2000
	DNA36638-1056	21	21	209456	November 12, 1997
30	DNA38260-1180	236	269	209397	October 17, 1997
	DNA40592-1242	303	344	209492	November 21, 1997
	DNA41374-1312	294	333	-------	----------
	DNA44194-1317	322	381	209808	April 28, 1998
	DNA53517-1366	388	720	209802	April 23, 1998
35	DNA53971-1359	435	866	209750	April 7, 1998
	DNA53987-1438	433	840	209858	May 12, 1998
	DNA57700-1408	483	982	203583	January 12, 1999
	DNA59620-1463	545	836	209989	June 16, 1998
	DNA60627-1508	589	1159	203092	August 4, 1998
40	DNA64890-1612	707	1358	203131	August 18, 1998

DNA66659-1593	685	1325	203269	September 22, 1998
DNA66667-1596	693	1338	203267	September 22, 1998
DNA68818-2536	739	1434	203657	February 9, 1999
DNA84210-2576	1888	4333	203818	March 2, 1999
DNA92218-2554	1866	4302	203834	March 9, 1999
DNA96878-2626	1947	4430	23-PTA	May 5, 1999
DNA98853-1739	2448	5727	203906	April 6, 1999

These deposits was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC 122 and the Commissioner's rules pursuant thereto (including 37 CFR 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

What is claimed:

1. A composition useful for the treatment of immune related diseases, comprising a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide, agonist or fragment thereof and a carrier or excipient, having the properties of:

(a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof,

(b) stimulating or enhancing an immune response in a mammal in need thereof, or

(c) increasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen.

2. The composition of claim 1 comprising an effective amount of a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide, agonist, antagonist or fragment thereof.

3. The composition of claim 2 further comprising a growth inhibitory agent, cytotoxic agent or chemotherapeutic agent.

4. Use of a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide, agonist or a fragment thereof to prepare a composition having the properties of:

(a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof,

(b) stimulating or enhancing an immune response in a mammal in need thereof, or

(c) increasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen.

5. The use of claims 4 comprising an effective amount of a PRO200, PRO204, PRO212,

PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430, PRO5727 polypeptide, agonist, antagonist or fragment thereof.

6. The composition of claim 2 further comprising a growth inhibitory agent, cytotoxic agent or chemotherapeutic agent.

7. A method of treating an immune related disorder, such as a T cell mediated disorder, in a mammal in need thereof, comprising administering to the mammal an effective amount of a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide, an agonist antibody thereof, an antagonist antibody thereto, or a fragment thereof.

8. The method of claim 7, wherein the disorder is selected from systemic lupus erythematosis, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis, idiopathic inflammatory myopathies, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease.

9. The composition or use of any of the preceding claims, wherein the agonist or antagonist is a monoclonal antibody.

10. The composition or use of any of the preceding claims, wherein the agonist or antagonist is an antibody fragment or a single-chain antibody.

11. The composition or use of claims 9 or 10, wherein the antibody has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues.

12. A method for determining the presence of a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430, PRO5727 polypeptide, comprising exposing a cell suspected of containing the polypeptide to an anti- PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430, anti-PRO5727 antibody, respectively, and determining binding of the antibody to the cell.

13. A method of diagnosing an immune related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

14. A method of diagnosing an immune related disease in a mammal, comprising (a) contacting an anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-

PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and the polypeptide in the test sample.

15. An immune related disease diagnostic kit, comprising an anti-PRO200, anti-PRO204, anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363, anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody or fragment thereof and a carrier in suitable packaging.

16. The kit of claim 15, further comprising instructions for using the antibody to detect a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide.

17. An article of manufacture, comprising:

a container;

an instruction on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for inhibiting or reducing an immune response in a mammal, the instruction on the container indicates that the composition can be used for treating an immune related disease, and the active agent in the composition is an agent inhibiting the expression and/or activity of a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358,

PRO1325. PRO1338. PRO1434, PRO4333. PRO4302, PRO4430 or PRO5727 polypeptide.

18. The article of manufacture of claim 17 wherein said active agent is an anti-PRO200, anti-PRO204. anti-PRO212, anti-PRO216, anti-PRO226, anti-PRO240, anti-PRO235, anti-PRO245, anti-PRO172, anti-PRO273, anti-PRO272, anti-PRO332, anti-PRO526, anti-PRO701, anti-PRO361, anti-PRO362, anti-PRO363. anti-PRO364, anti-PRO356, anti-PRO531, anti-PRO533, anti-PRO1083, anti-PRO865, anti-PRO770, anti-PRO769, anti-PRO788, anti-PRO1114, anti-PRO1007, anti-PRO1184, anti-PRO1031, anti-PRO1346, anti-PRO1155, anti-PRO1250, anti-PRO1312, anti-PRO1192, anti-PRO1246, anti-PRO1283, anti-PRO1195, anti-PRO1343, anti-PRO1418, anti-PRO1387, anti-PRO1410, anti-PRO1917, anti-PRO1868, anti-PRO205, anti-PRO21, anti-PRO269, anti-PRO344, anti-PRO333, anti-PRO381, anti-PRO720, anti-PRO866, anti-PRO840, anti-PRO982, anti-PRO836, anti-PRO1159, anti-PRO1358, anti-PRO1325, anti-PRO1338, anti-PRO1434, anti-PRO4333, anti-PRO4302, anti-PRO4430 or anti-PRO5727 antibody.

19. A method for identifying a compound capable of inhibiting the expression or activity of a PRO200. PRO204. PRO212. PRO216. PRO226. PRO240. PRO235. PRO245. PRO172. PRO273. PRO272. PRO332. PRO526. PRO701. PRO361. PRO362. PRO363. PRO364, PRO356. PRO531. PRO533. PRO1083. PRO865. PRO770. PRO769. PRO788. PRO1114. PRO1007. PRO1184. PRO1031. PRO1346. PRO1155. PRO1250. PRO1312. PRO1192. PRO1246. PRO1283. PRO1195. PRO1343. PRO1418. PRO1387. PRO1410. PRO1917. PRO1868. PRO205. PRO21. PRO269. PRO344. PRO333. PRO381. PRO720. PRO866. PRO840. PRO982. PRO836. PRO1159. PRO1358. PRO1325. PRO1338. PRO1434. PRO4333. PRO4302. PRO4430 or PRO5727 polypeptide, comprising contacting a candidate compound with the polypeptide under conditions and for a time sufficient to allow these two components to interact.

20. The method of claim 19. wherein the candidate compound or the PRO200. PRO204. PRO212. PRO216. PRO226. PRO240. PRO235. PRO245. PRO172. PRO273. PRO272. PRO332. PRO526. PRO701. PRO361. PRO362. PRO363. PRO364. PRO356. PRO531. PRO533. PRO1083. PRO865. PRO770. PRO769. PRO788. PRO1114. PRO1007. PRO1184. PRO1031. PRO1346. PRO1155. PRO1250. PRO1312. PRO1192. PRO1246. PRO1283. PRO1195. PRO1343. PRO1418. PRO1387. PRO1410. PRO1917. PRO1868. PRO205. PRO21. PRO269. PRO344. PRO333. PRO381. PRO720. PRO866. PRO840. PRO982. PRO836. PRO1159. PRO1358. PRO1325. PRO1338. PRO1434. PRO4333. PRO4302. PRO4430 or PRO5727 polypeptide is immobilized on a solid support.

21. The method of claim 20. wherein the non-immobilized component carries a detectable label.

22. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:11), Figure 5 (SEQ ID NO:13), Figure 7 (SEQ ID NO:18), Figure 9 (SEQ ID NO:20), Figure 11 (SEQ ID NO:25), Figure 13 (SEQ ID NO:30), Figure 15 (SEQ ID NO:35), Figure 17 (SEQ ID NO:40), Figure 19 (SEQ ID NO:45), Figure 21 (SEQ ID NO:50), Figure 23 (SEQ ID NO:56), Figure 25 (SEQ ID NO:61), Figure 27 (SEQ ID NO:66), Figure 29 (SEQ ID NO:71), Figure

31 (SEQ ID NO:79), Figure 33 (SEQ ID NO:86), Figure 35 (SEQ ID NO:91), Figure 37 (SEQ ID NO:101), Figure 39 (SEQ ID NO:106), Figure 41 (SEQ ID NO:111), Figure 43 (SEQ ID NO:116), Figure 45 (SEQ ID NO:123), Figure 47 (SEQ ID NO:133), Figure 49 (SEQ ID NO:139), Figure 51 (SEQ ID NO:141), Figure 53 (SEQ ID NO:143), Figure 55 (SEQ ID NO:145), Figure 57 (SEQ ID NO:147), Figure 59 (SEQ ID NO:149), Figure 61 (SEQ ID NO:151), Figure 63 (SEQ ID NO:156), Figure 65 (SEQ ID NO:158), Figure 67 (SEQ ID NO:160), Figure 69 (SEQ ID NO:162), Figure 71 (SEQ ID NO:167), Figure 73 (SEQ ID NO:169), Figure 75 (SEQ ID NO:177), Figure 77 (SEQ ID NO:179), Figure 79 (SEQ ID NO:184), Figure 81 (SEQ ID NO:186), Figure 83 (SEQ ID NO:188), Figure 85 (SEQ ID NO:190), Figure 87 (SEQ ID NO:192), Figure 89 (SEQ ID NO:228), Figure 91 (SEQ ID NO:230), Figure 93 (SEQ ID NO:232), Figure 95 (SEQ ID NO:240), Figure 97 (SEQ ID NO:248), Figure 99 (SEQ ID NO:250), Figure 101 (SEQ ID NO:255), Figure 103 (SEQ ID NO:257), Figure 105 (SEQ ID NO:266), Figure 107 (SEQ ID NO:268), Figure 109 (SEQ ID NO:270), Figure 111 (SEQ ID NO:272), Figure 113 (SEQ ID NO:274), Figure 115 (SEQ ID NO:276), Figure 117 (SEQ ID NO:278), Figure 119 (SEQ ID NO:280), Figure 121 (SEQ ID NO:285), Figure 123 (SEQ ID NO:292), Figure 125 (SEQ ID NO:294) or Figure 127 (SEQ ID NO:296.

23. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:11), Figure 5 (SEQ ID NO:13), Figure 7 (SEQ ID NO:18), Figure 9 (SEQ ID NO:20), Figure 11 (SEQ ID NO:25), Figure 13 (SEQ ID NO:30), Figure 15 (SEQ ID NO:35), Figure 17 (SEQ ID NO:40), Figure 19 (SEQ ID NO:45), Figure 21 (SEQ ID NO:50), Figure 23 (SEQ ID NO:56), Figure 25 (SEQ ID NO:61), Figure 27 (SEQ ID NO:66), Figure 29 (SEQ ID NO:71), Figure 31 (SEQ ID NO:79), Figure 33 (SEQ ID NO:86), Figure 35 (SEQ ID NO:91), Figure 37 (SEQ ID NO:101), Figure 39 (SEQ ID NO:106), Figure 41 (SEQ ID NO:111), Figure 43 (SEQ ID NO:116), Figure 45 (SEQ ID NO:123), Figure 47 (SEQ ID NO:133), Figure 49 (SEQ ID NO:139), Figure 51 (SEQ ID NO:141), Figure 53 (SEQ ID NO:143), Figure 55 (SEQ ID NO:145), Figure 57 (SEQ ID NO:147), Figure 59 (SEQ ID NO:149), Figure 61 (SEQ ID NO:151), Figure 63 (SEQ ID NO:156), Figure 65 (SEQ ID NO:158), Figure 67 (SEQ ID NO:160), Figure 69 (SEQ ID NO:162), Figure 71 (SEQ ID NO:167), Figure 73 (SEQ ID NO:169), Figure 75 (SEQ ID NO:177), Figure 77 (SEQ ID NO:179), Figure 79 (SEQ ID NO:184), Figure 81 (SEQ ID NO:186), Figure 83 (SEQ ID NO:188), Figure 85 (SEQ ID NO:190), Figure 87 (SEQ ID NO:192), Figure 89 (SEQ ID NO:228), Figure 91 (SEQ ID NO:230), Figure 93 (SEQ ID NO:232), Figure 95 (SEQ ID NO:240), Figure 97 (SEQ ID NO:248), Figure 99 (SEQ ID NO:250), Figure 101 (SEQ ID NO:255), Figure 103 (SEQ ID NO:257), Figure 105 (SEQ ID NO:266), Figure 107 (SEQ ID NO:268), Figure 109 (SEQ ID NO:270), Figure 111 (SEQ ID NO:272), Figure 113 (SEQ ID NO:274), Figure 115 (SEQ ID NO:276), Figure 117 (SEQ ID NO:278), Figure 119 (SEQ ID NO:280), Figure 121 (SEQ ID NO:285), Figure 123 (SEQ ID NO:292), Figure 125 (SEQ ID NO:294) or Figure 127 (SEQ ID NO:296.

24. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the full-length coding sequence of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:11), Figure 5 (SEQ ID NO:13), Figure 7 (SEQ ID NO:18), Figure 9 (SEQ ID NO:20), Figure 11 (SEQ ID NO:25), Figure 13 (SEQ ID NO:30), Figure 15 (SEQ

ID NO:35), Figure 17 (SEQ ID NO:40), Figure 19 (SEQ ID NO:45), Figure 21 (SEQ ID NO:50), Figure 23 (SEQ ID NO:56), Figure 25 (SEQ ID NO:61), Figure 27 (SEQ ID NO:66), Figure 29 (SEQ ID NO:71), Figure 31 (SEQ ID NO:79), Figure 33 (SEQ ID NO:86), Figure 35 (SEQ ID NO:91), Figure 37 (SEQ ID NO:101), Figure 39 (SEQ ID NO:106), Figure 41 (SEQ ID NO:111), Figure 43 (SEQ ID NO:116), Figure 45 (SEQ ID NO:123), Figure 47 (SEQ ID NO:133), Figure 49 (SEQ ID NO:139), Figure 51 (SEQ ID NO:141), Figure 53 (SEQ ID NO:143), Figure 55 (SEQ ID NO:145), Figure 57 (SEQ ID NO:147), Figure 59 (SEQ ID NO:149), Figure 61 (SEQ ID NO:151), Figure 63 (SEQ ID NO:156), Figure 65 (SEQ ID NO:158), Figure 67 (SEQ ID NO:160), Figure 69 (SEQ ID NO:162), Figure 71 (SEQ ID NO:167), Figure 73 (SEQ ID NO:169), Figure 75 (SEQ ID NO:177), Figure 77 (SEQ ID NO:179), Figure 79 (SEQ ID NO:184), Figure 81 (SEQ ID NO:186), Figure 83 (SEQ ID NO:188), Figure 85 (SEQ ID NO:190), Figure 87 (SEQ ID NO:192), Figure 89 (SEQ ID NO:228), Figure 91 (SEQ ID NO:230), Figure 93 (SEQ ID NO:232), Figure 95 (SEQ ID NO:240), Figure 97 (SEQ ID NO:248), Figure 99 (SEQ ID NO:250), Figure 101 (SEQ ID NO:255), Figure 103 (SEQ ID NO:257), Figure 105 (SEQ ID NO:266), Figure 107 (SEQ ID NO:268), Figure 109 (SEQ ID NO:270), Figure 111 (SEQ ID NO:272), Figure 113 (SEQ ID NO:274), Figure 115 (SEQ ID NO:276), Figure 117 (SEQ ID NO:278), Figure 119 (SEQ ID NO:280), Figure 121 (SEQ ID NO:285), Figure 123 (SEQ ID NO:292), Figure 125 (SEQ ID NO:294) or Figure 127 (SEQ ID NO:296.

25. Isolated nucleic acid having at least 80% nucleic acid sequence identity to the full-length coding sequence of the DNA deposited under ATCC accession number 209653, 209380, 209254, 209381, 209376, 209260, 209374, 209265, 209419, 209424, 209388, 209433, 209704, 209720, 209621, 209620, 209616, 209436, 209422, 209702, 209480, 209859, 209774, 209801, 209802, 209849, 209905, 209950, 209962, 209866, 203128, 209986, 203173, 203132, 203093, 203457, 203244, 203094, 203282, 203276, 203160, 203277, 203573, 203553, ------, 209456, 209397, 209492, ------, 209808, 209802, 209750, 209858, 203583, 209989, 203092, 203131, 203269, 203267, 203657, 203818, 203834, 23-PTA, 203906.

26. A vector comprising the nucleic acid of any one of Claims 22 to 25.

27. The vector of Claim 26 operably linked to control sequences recognized by a host cell transformed with the vector.

28. A host cell comprising the vector of Claim 26.

29. The host cell of Claim 28, wherein said cell is a CHO cell.

30. The host cell of Claim 28, wherein said cell is an *E. coli*.

31. The host cell of Claim 28, wherein said cell is a yeast cell.

32. A process for producing a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363,

PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide comprising culturing the host cell of Claim 28 under conditions suitable for expression of said polypeptide and recovering said polypeptide from the cell culture.

33. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124), Figure 48 (SEQ ID NO:134), Figure 50 (SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297).

34. An isolated polypeptide scoring at least 80% positives when compared to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124), Figure 48 (SEQ ID NO:134), Figure 50 (SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID

NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297).

35. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence encoded by the full-length coding sequence of the DNA deposited under ATCC accession number 209653, 209380, 209254, 209381, 209376, 209260, 209374, 209265, 209419, 209424, 209388, 209433, 209704, 209720, 209621, 209620, 209616, 209436, 209422, 209702, 209480, 209859, 209774, 209801, 209802, 209849, 209905, 209950, 209962, 209866, 203128, 209986, 203173, 203132, 203093, 203457, 203244, 203094, 203282, 203276, 203160, 203277, 203573, 203553, ------, 209456, 209397, 209492, ------, 209808, 209802, 209750, 209858, 203583, 209989, 203092, 203131, 203269, 203267, 203657, 203818, 203834, 23-PTA, 203906.

36. A chimeric molecule comprising a polypeptide according to any one of Claims 33 to 35 fused to a heterologous amino acid sequence.

37. The chimeric molecule of Claim 36, wherein said heterologous amino acid sequence is an epitope tag sequence.

38. The chimeric molecule of Claim 36, wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

39. An antibody which specifically binds to a polypeptide according to any one of Claims 33 to 35.

40. The antibody of Claim 39, wherein said antibody is a monoclonal antibody, a humanized antibody or a single-chain antibody.

41. Isolated nucleic acid having at least 80% nucleic acid sequence identity to:

(a) a nucleotide sequence encoding the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID

NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124), Figure 48 (SEQ ID NO:134), Figure 50 (SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297) lacking its associated signal peptide;

(b) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124), Figure 48 (SEQ ID NO:134), Figure 50 (SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297) with its associated signal peptide; or

(c) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124),

Figure 48 (SEQ ID NO:134), Figure 50 (SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297) lacking its associated signal peptide.

42. An isolated polypeptide having at least 80% amino acid sequence identity to:

(a) the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124), Figure 48 (SEQ ID NO:134), Figure 50 (SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297), lacking its associated signal peptide;

(b) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124), Figure 48 (SEQ ID NO:134), Figure 50

(SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297), with its associated signal peptide; or

(c) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:12), Figure 6 (SEQ ID NO:14), Figure 8 (SEQ ID NO:19), Figure 10 (SEQ ID NO:21), Figure 12 (SEQ ID NO:26), Figure 14 (SEQ ID NO:31), Figure 16 (SEQ ID NO:36), Figure 18 (SEQ ID NO:41), Figure 20 (SEQ ID NO:46), Figure 22 (SEQ ID NO:51), Figure 24 (SEQ ID NO:57), Figure 26 (SEQ ID NO:62), Figure 28 (SEQ ID NO:67), Figure 30 (SEQ ID NO:72), Figure 32 (SEQ ID NO:80), Figure 34 (SEQ ID NO:87), Figure 36 (SEQ ID NO:92), Figure 38 (SEQ ID NO:102), Figure 40 (SEQ ID NO:107), Figure 42 (SEQ ID NO:112), Figure 44 (SEQ ID NO:117), Figure 46 (SEQ ID NO:124), Figure 48 (SEQ ID NO:134), Figure 50 (SEQ ID NO:140), Figure 52 (SEQ ID NO:142), Figure 54 (SEQ ID NO:144), Figure 56 (SEQ ID NO:146), Figure 58 (SEQ ID NO:148), Figure 60 (SEQ ID NO:150), Figure 62 (SEQ ID NO:152), Figure 64 (SEQ ID NO:157), Figure 66 (SEQ ID NO:159), Figure 68 (SEQ ID NO:161), Figure 70 (SEQ ID NO:163), Figure 72 (SEQ ID NO:168), Figure 74 (SEQ ID NO:170), Figure 76 (SEQ ID NO:178), Figure 78 (SEQ ID NO:180), Figure 80 (SEQ ID NO:185), Figure 82 (SEQ ID NO:187), Figure 84 (SEQ ID NO:189), Figure 86 (SEQ ID NO:191), Figure 88 (SEQ ID NO:193), Figure 90 (SEQ ID NO:229), Figure 92 (SEQ ID NO:231), Figure 94 (SEQ ID NO:233), Figure 96 (SEQ ID NO:241), Figure 98 (SEQ ID NO:249), Figure 100 (SEQ ID NO:251), Figure 102 (SEQ ID NO:256), Figure 104 (SEQ ID NO:258), Figure 106 (SEQ ID NO:267), Figure 108 (SEQ ID NO:269), Figure 110 (SEQ ID NO:271), Figure 112 (SEQ ID NO:273), Figure 114 (SEQ ID NO:275), Figure 116 (SEQ ID NO:277), Figure 118 (SEQ ID NO:279), Figure 120 (SEQ ID NO:281), Figure 122 (SEQ ID NO:286), Figure 124 (SEQ ID NO:293), Figure 126 (SEQ ID NO:295) or Figure 128 (SEQ ID NO:297), lacking its associated signal peptide.

43. A method of affecting the proliferation of T-cells comprising contacting PBMC cells with an effective amount of a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide and measuring the change in proliferation from control levels.

44. A method of affecting vascular permeability comprising injecting a test animal with an effective amount of a PRO200, PRO204, PRO212, PRO216, PRO226, PRO240, PRO235, PRO245, PRO172, PRO273, PRO272, PRO332, PRO526, PRO701, PRO361, PRO362, PRO363, PRO364, PRO356, PRO531, PRO533, PRO1083, PRO865, PRO770, PRO769, PRO788, PRO1114, PRO1007, PRO1184, PRO1031, PRO1346, PRO1155, PRO1250, PRO1312, PRO1192, PRO1246, PRO1283, PRO1195, PRO1343, PRO1418, PRO1387, PRO1410, PRO1917, PRO1868, PRO205, PRO21, PRO269, PRO344, PRO333, PRO381, PRO720, PRO866, PRO840, PRO982, PRO836, PRO1159, PRO1358, PRO1325, PRO1338, PRO1434, PRO4333, PRO4302, PRO4430 or PRO5727 polypeptide, and measuring the resulting extent of vascular permeability.